Development and validation of a clinical prediction tool for the diagnosis of tuberculous meningitis

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DEDICATION

This research is dedicated to the participants, without whom this research would not be possible.

ABSTRACT

Introduction: Tuberculous (TB) meningitis is the most lethal and disabling form of TB. A disproportionate burden of TB meningitis is in resource-limited settings. There is considerable variation in mortality and neurological sequelae reported for TB meningitis across available studies, the reasons for which remain unclear. Delayed diagnosis and treatment, which is a risk factor for poor outcomes, is caused in part by lack of availability of diagnostic tests that are both rapid and accurate. Several attempts have been made to develop clinical prediction tools to fill this gap, but none have performed sufficiently well to be broadly implemented.

Purpose: We aimed to (1) ascertain heterogeneity in TB meningitis outcomes; (2) develop and validate a clinical prediction tool for diagnosing TB meningitis; and (3) externally validate this clinical prediction tool to determine the overall accuracy of classification.

Methods: We conducted two systematic reviews: one to identify studies reporting TB meningitis mortality and neurological sequelae and another to identify studies that undertook diagnostic testing for TB meningitis to obtain individual participant data (IPD) from. From the first systematic review, we conducted a meta-analysis of TB meningitis mortality and neurological sequelae from studies that met the inclusion criteria. We assessed heterogeneity in mortality by conducting stratified analyses by time of reported outcome, HIV status, geographic location, and year published. From the second systematic review, we contacted the authors and attained permission to use IPD from studies that met the inclusion criteria. We harmonized the data and imputed for missing values when possible. Three multivariate prediction model (MPM) development

strategies were employed to develop the clinical prediction tool for TB meningitis cases. First, an IPD meta-analysis using a logistic regression MPM with stratified intercepts for each country was fitted with key predictors. Then, we developed classification and regression tree (CART) and random forest MPMs with machine learning methods. All three MPMs were internally validated and assessed for performance using all available data in a k-fold internal-external cross-validation (IECV) approach. In our final analysis, we externally validated all three MPMs in a dataset that was not used in the development stage.

Results: In our first systematic review and meta-analysis, pooled six-month mortality was 24% and showed significant heterogeneity (I^2 >95%; p<0.01). Physical disability was reported in 32% (95%CI; 22-43%) of TB meningitis survivors. The heterogeneity in mortality was partly explained by HIV status and geographic location. Mortality ranged from 2% to 67% in Asian studies and from 23% to 80% in sub-Saharan African studies. Mortality was significantly worse in HIV-positive persons and in persons from studies conducted in sub-Saharan Africa. In our second systematic review, we identified and obtained IPD from 15 studies with a total of 3,671 individual participants. All three MPMs indicated cerebrospinal fluid (CSF) white blood cell (WBC) count, WBC differential, CSF glucose, CSF cryptococcal antigen, and blood glucose as significant predictors of TB meningitis. IECV revealed significant heterogeneity in performance between IPD studies, which varied based on the prevalence of HIV in the IPD study. Overall, the machine learning MPMs were not superior in performance to the logistic MPM; however, random forest performed slightly better than the logistic MPM. In external validation, the logistic MPM outperformed both CART and random forest.

Discussion: Results from these studies indicate the significant contribution HIV co-infection has on outcomes and clinical prediction tool performance for TB meningitis. MPMs based on clinical and lab values more readily accessible in resource-limited settings yield well-performing clinical prediction tools. The logistic MPM had the best performance and external validity in an HIV-prevalent setting for TB meningitis.

Conclusion: Heterogeneity in TB meningitis outcomes and diagnostic performance persist. HIV-status and geographic location are major contributors to variation in TB meningitis outcomes. We were successful in developing a model that can better account for this heterogeneity. The logistic MPM poses a generalizable clinical prediction tool with the potential to reduce the delay in diagnosis, and subsequent poor outcomes, in TB meningitis.

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LIST OF ABBREVIATIONS

- TB : Tuberculosis or Tuberculous
- HIV : Human Immunodeficiency Virus
- ART : Antiretroviral therapy
- WHO : World Health Organization
- CSF : Cerebrospinal fluid
- WBC : White blood cell
- MPM : Multivariable prediction model
- UTBMCD : Uniform TB Meningitis Case Definition
- NAATs : Nucleic acid amplification tests
- CPT : Clinical prediction tool
- DNA : Dioxyribonucleic acid
- MRC : Medical Research Council
- IPD : Individual participant data
- IECV : Internal-external cross-validation
- CrAg : Cryptococcal antigen
- ROC : Receiver operating characteristic

CHAPTER 1. OVERVIEW OF TUBERCULOUS MENINGITIS DIAGNOSTICS

This dissertation aims to improve the diagnostic strategy for tuberculous (TB) meningitis. In Manuscript 1, we aim to ascertain heterogeneity in TB meningitis mortality and neurological sequelae via systematic review and meta-analysis. In Manuscript 2, we aim to build a clinical prediction tool using machine learning methods. Our goal is to address the underlying heterogeneity observed in Manuscript 1 as well as account for case-mix variation so that the clinical prediction tool is built to perform well in any population or setting. For Manuscript 3, we aim to externally validate the clinical prediction tool developed in Manuscript 2 in a population in Uganda. This chapter describes the epidemiology of TB meningitis, the context of TB meningitis diagnostic challenges, clinical prediction tools, and machine learning methods.

Epidemiology of Tuberculous Meningitis

Tuberculosis (TB) is a disease caused by the infection of the bacillus *Mycobacterium tuberculosis*.[1] *M. tuberculosis* is spread by small airborne droplets generated when a person with pulmonary TB coughs, sneezes, or otherwise expels bacteria into the air.[1] These droplets can remain airborne for up to a few hours after expectoration. Introduction of *M. tuberculosis* into the lungs leads to infection of the respiratory system (pulmonary TB) but the infection can also spread to other sites in the body (extrapulmonary TB) including the brain and central nervous system.[1] TB meningitis is caused when *M. tuberculosis* enters the cerebrospinal fluid (CSF), leading to inflammation of the meninges, causing meningitis.[2]

Globally, 10 million cases of TB were reported in 2019, and TB is currently the leading cause of mortality from a single infectious agent worldwide.[1] According to the World Health Organization (WHO), about a quarter of the world's population is infected with *M. tuberculosis*.[1] TB is a disease of poverty, economic distress, vulnerability, and marginalization. Consequently, 90% of cases are derived from 30 high-TB burden countries.[1] TB meningitis accounts for 1-5% of global TB cases, and at least 100,000 cases are estimated annually.[3] The proportion of TB meningitis in people living with TB varies considerably by TB prevalence, age, and HIV. HIV co-infection is the most significant risk factor for TB meningitis in adults.[4] However, our understanding of the global burden of TB meningitis is poor. Many cases of TB meningitis remain undiagnosed due to inadequate diagnostic test performance and lack of available testing availability, so population-based estimates of TB meningitis incidence are often not reported and challenging to determine.[3,4]

Although TB meningitis disproportionately impacts resource-limited settings, TB meningitis accounts for the highest proportion of TB deaths in both resource-rich and -limited settings.[3] TB meningitis is the most devastating form of TB, with an estimated mortality of 16% (95% Confidence Interval (CI): 10-24%) among HIV-negative patients, and 57% (95% CI; 48-67%) among HIV-positive patients.[3,5] In the absence of treatment, TB meningitis is uniformly fatal.[4] Up to 50% of survivors suffer from long-term neurologic sequelae such as blindness, stroke, and seizures.[4] Delays in diagnosis and treatment initiation are major contributing factors to the high morbidity and mortality in TB meningitis patients, particularly in resource-limited settings.[3,4]

Challenges in Tuberculous Meningitis Diagnostics

Identifying the etiology of meningitis is challenging since many of the discriminating clinical symptoms are often nonspecific and subacute.[2,6] Adults with TB meningitis commonly present with stiff neck, headache, fever and vomiting, which are symptoms commonly seen with other forms of bacterial meningitis.[6] However, patients with TB meningitis typically report longer symptom duration—up to 1 month–with symptoms that are consistent with pulmonary TB infection such as cough, night sweats, and weight loss.[6,7] TB meningitis presentation is also characterized by a higher likelihood of neurologic symptoms such as altered mental status, personality changes, cranial nerve neuropathy (typically cranial nerve IV), and coma.[7] Routine evaluation of cerebrospinal fluid (CSF) may help elucidate meningitis etiology. TB meningitis is generally thought of as causing an increase in lymphocytic white blood cell (WBC) count with elevated protein and low glucose in CSF.[8] However, there is no typical pattern of CSF values that can reliably distinguish TB meningitis from other meningitis etiologies, particularly in the presence of HIV co-infection.[8]

Diagnostic tests aimed at identifying *M. tuberculosis* in CSF are lengthy, have poor diagnostic performance, and/or unavailable in resource-limited settings. While recently introduced nucleic acid amplification tests (NAATs) that test for the DNA of TB have the potential to improve timeliness of diagnosis, the moderate sensitivity (50-70%) means that negative tests cannot fully exclude disease.[9] This is largely attributable to the pauci-bacillary nature of TB meningitis. Due to significant inflammation in the brain and subsequent poor CSF circulation, it is possible to have TB meningitis with very low or no *M. tuberculosis* in the CSF.[8] Diagnostic approaches

that rely on microbiologic confirmation will inevitably miss cases of TB meningitis, further delaying treatment. Delayed treatment is the main risk factor for death for TB meningitis.[3] Thus, diagnostic approaches for TB meningitis must include a combination of microbiologic and clinical assessments that can better encompass pauci-bacillary and bacillary case types of TB meningitis.

A major stumbling block in TB meningitis research had been the absence of a single reference standard test or criteria for the diagnosis of TB meningitis. In 2010, a committee of 41 international TBM experts developed a standardized criteria, known as a case definition, for the diagnosis of TB meningitis to use in future clinical research called the Uniform TB Meningitis Case Definition (UTBMCD) (**Table 1.1**).[10] The UTBMCD has helped to standardize research, but it cannot be used as an immediate clinical diagnostic tool because it depends on delayed results, such as mycobacterial culture, which can take up to 8 weeks for growth to occur.[3] The UTBMCD also includes brain imaging to define the diagnosis of TB

Table 1.1. Uniform TBM Case Definition

Criteria	Diagnostic Score
Clinical criteria (maximum category score = 6)	
Symptom duration of >5 d	4
Systemic symptoms suggestive of tuberculosis (≥1): weight loss/(poor weight gain in children), night sweats, or persistent cough >2 wk	2
History of recent close contact with an individual with pulmonary tuberculosis or a positive TST/IGRA in a child aged <10 y	2
Focal neurological deficit (excluding cranial nerve palsies)	1
Cranial nerve palsy	1
CSF criteria (maximum category score = 4)	
Clear appearance	1
Cells: 10–500/μL	1
Lymphocytic predominance (>50%)	1
Protein concentration >1 g/L	1
CSF: plasma glucose ratio of <50% or an absolute CSF glucose concentration <2.2 mmol/L	1
Cerebral imaging criteria (maximum category score = 6)	
Hydrocephalus (CT and/or MRI)	1
Basal meningeal enhancement (CT and/or MRI)	2
Tuberculoma (CT and/or MRI)	2
Infarct (CT and/or MRI)	1
Precontrast basal hyperdensity (CT)	2
Evidence of tuberculosis elsewhere (maximum category score = 4)	
Chest radiograph suggestive of active tuberculosis (excludes miliary tuberculosis)	2
Chest radiograph suggestive of miliary tuberculosis	4
CT/MRI/US evidence for tuberculosis outside the CNS	2
AFB identified or *Mycobacterium tuberculosis* cultured from another source, ie, sputum, lymph node, gastric washing, urine, blood culture	4
Exclusion of alternative diagnoses: an alternative diagnosis must be confirmed microbiologically, serologically, or histopathologically	
Definite TBM = AFB seen on CSF microscopy, positive CSF *M. tuberculosis* culture, or positive CSF *M. tuberculosis* commercial NAAT in the setting of symptoms/signs suggestive of meningitis; or AFB seen in the context of histological changes consistent with tuberculosis brain or spinal cord together with suggestive symptoms/signs and CSF changes, or visible meningitis (on autopsy).	
Probable TBM = total score of ≥12 when neuroimaging available = total score of ≥10 when neuroimaging unavailable	
Possible TBM = total score of 6–11 when neuroimaging available = total score of 6–9 when neuroimaging unavailable	

Source: Marais et al [12].

Abbreviations: AFB, acid-fast bacilli; CNS, central nervous system; CSF, cerebrospinal fluid; CT, computed tomography; IGRA, interferon-γ release assay; MRI, magnetic resonance imaging; NAAT, nucleic acid amplification test; TBM, tuberculous meningitis; TST, tuberculin skin test; US, ultrasound.

meningitis, which is not available in many resource-limited settings.[3] Having a rapid diagnostic test ensures that the patient can begin treatment at the earliest possible time. Due to the brain damage that TB meningitis causes and high risk of long-term disability and death, TB meningitis is a medical emergency.[3] Availability of a diagnostic method that facilitates early diagnosis and prompt treatment could save lives and reduce neurologic disability in survivors. Improving the strategies for rapid and efficient TB meningitis diagnosis is an urgent need.[3]

Multivariate Prediction Models & Clinical Prediction Tools

Another approach to improving rapid TB meningitis diagnosis, particularly in resource-limited settings, is to develop a clinical prediction tool based on a multivariate prediction model (MPM). A MPM is a mathematical algorithm based on several inputs, or variables, formulated with the aid of statistical modeling that predicts the probability of having a disease. The statistical modeling determines which criteria are most predictive of the presence of a disease, typically patient characteristics (such as age or sex) or biological markers found in human specimens that can be measured in a clinical laboratory.

The advantage of a MPM is that the prediction of TB meningitis is based on patient characteristics and biological markers that differentiate TB meningitis from other types of meningitis. This is a novel approach to the diagnosis of TB meningitis that overcomes the challenge of prior diagnostic methods, such as the UTBMCD and NAATs, which focus on identifying the pathogen.

At least ten clinical prediction tools have been published for the diagnosis of TB meningitis (see **Table 1.2**).

Table 1.2. Published Clinical Prediction Tools for TB Meningitis

Citation	Population	Case comparison	Predictors of TB Meningitis
Kumar [11]	Children from India	TBM versus other meningitis	Symptoms ≥7 days Optic atrophy Focal neurological deficit Extrapyramidal movements CSF leukocytes <50% neutrophils
Thwaites [12]	Adults from Vietnam	TBM versus bacterial meningitis	Age <36 years Blood leukocytes <15 × 109/l Symptoms ≥6 days CSF leukocytes <750/mm^3 CSF neutrophils <90%
Youssef.[13]	Children and adults from Egypt	TBM versus bacterial meningitis	Symptoms >5 days CSF leukocytes <1000/mm^3 Clear CSF CSF lymphocytes >30% CSF protein >100 mg/L
Cohen[14]	Adults from Malawi (77% HIV-positive)	TBM versus cryptococcal meningitis	Low CSF opening pressure Neck stiffness Raised CSF leukocytes Low Glasgow Coma Scale score High fever
Patel[15]	Adults from South Africa (84% HIV+)	TBM versus other meningitis	CSF:blood glucose ratio ≤0.2 CSF lymphocytes >200/mm^3 CD4+ cell count <200 × 106/L Negative cryptococcal antigen test
Hristea[16]	Adults from Turkey	TBM versus viral meningitis	Symptoms ≥5 days MRC grade II or III CSF:blood glucose ratio <0.5 CSF protein >100 mg/dL
Vibha[17]	Adults from India	TBM versus bacterial meningitis	Living in a rural area Symptoms ≥6 days Cranial nerve palsy Hemiplegia Clear CSF CSF neutrophils <75%
Dendane[18]	Adults from Morocco	TBM versus bacterial meningitis	Female sex Symptoms ≥0 days Focal neurological deficits Blood leukocytes <15 × 109/L Plasma sodium <130 mmol/L CSF leukocytes <400/mm^3
Zhang [19]	Adults from China (all HIV-uninfected)	TBM versus cryptococcal meningitis	Female sex Reduced consciousness No visual or hearing loss Evidence of extraneural tuberculosis CSF leukocytes ≥68/mm^3 CSF protein >0.91 mg/dL
Qamar [20]	Children from Pakistan	TBM versus bacterial meningitis	Hydrocephalus on brain CT CSF leukocytes <800/mm^3 CSF protein:glucose ratio ≥2

CSF, cerebrospinal fluid; MRC, Medical Research Council; TBM, tuberculous meningitis.

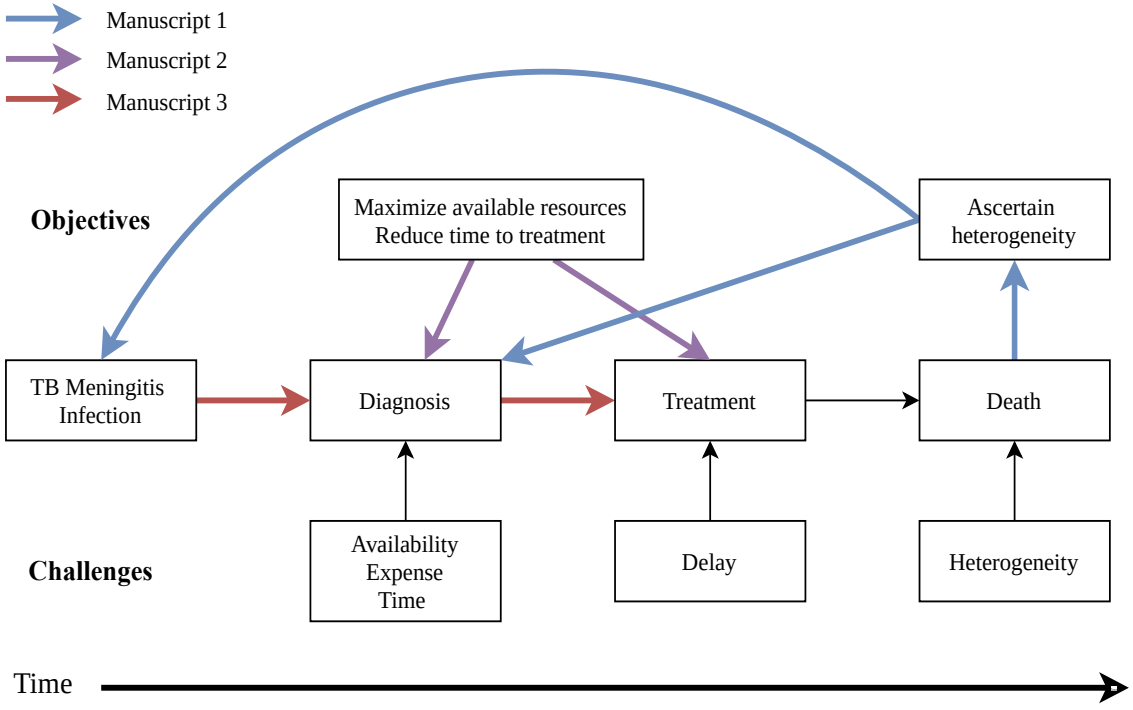
A major limitation of prior clinical prediction tools is that their performance is variable in different populations and settings. Only a few of these scoring systems have been externally validated.[3] External validation refers to the ability for a statistical model to perform well in a dataset or population separate from the dataset or population in which it was developed. Thus, if a tool or model is 'externally validated', then it performs well in any setting, population, or dataset. The primary contributing factor of varied model performance across different settings and populations is case-mix variation, which refers to the distribution of important population characteristics such as HIV status, age, and the population prevalence of TB. Case-mix variation across different settings can lead to genuine differences in the performance of a prediction model, even when the criteria truly predict the disease.[25] Prior clinical prediction tools were developed from a single site, including the tool developed by Thwaites et al.,[12] and therefore have not adequately accounted for case-mix variation. This impacts accuracy and clinical utility when applied to other populations and settings.

Conceptual Model: Diagnosis to Death

We have designed a conceptual model to illustrate the objectives of this dissertation, challenges in TB meningitis research, and pathways that we will explore in the three manuscripts (**Figure 1.1**). This conceptual model is based on the several key gaps in TB meningitis knowledge and research highlighted by experts in the TB Meningitis International Research Consortium.[2,3] The conceptual model depicts the timeline from TB meningitis infection to death. The challenges highlighted along the timeline are limited to the scope of this dissertation's objectives and are not exhaustive.

Manuscript 1, indicated by the blue arrows, examines the heterogeneity in outcomes of TB meningitis that inform important factors of case-mix variation, ultimately highlighting high-risk groups for infection, missed diagnosis, and death. Manuscript 2 aims to reduce the time between TB meningitis infection and diagnosis by using MPMs developed from a diverse dataset to develop a clinical prediction tool to improve diagnostics. In Manuscript 3, we will externally validate the clinical prediction tool developed in Manuscript 2 and ascertain diagnostic performance.

Figure 1.1. Dissertation Conceptual Model



CHAPTER 2. MANUSCRIPT 1: TUBERCULOSIS MENINGITIS MORTALITY

Introduction

In 2018, ten million cases of tuberculosis (TB) were reported globally;[26] and TB meningitis accounts for 1-5% of these cases.[27] TB meningitis is the most severe form of tuberculosis and is responsible for a considerable burden of neurological sequelae and mortality; a systematic review of treatment outcomes in 1,636 children with TB meningitis estimated 19.3% mortality.[28] There is considerable variation in the reported outcomes for adult TB meningitis across available studies, the reasons for which remain unclear. Two recent systematic reviews of adult TB meningitis outcomes reported substantial heterogeneity in mortality, with pooled estimates of 22.8% and 24.7%.[29,30] However, neither review attempted to explain the variation in treatment outcomes by stratifying studies by HIV status and geographical location. In addition, Wen and colleagues excluded all investigational treatment studies effectively excluding major treatment randomized controlled trials (RCTs) investigating regimens that have now become the standard of care (e.g., adjunctive steroids and delayed antiretroviral therapy (ART) for those with HIV-associated TB meningitis). Furthermore, there is a paucity of data in recent meta-analyses on drug resistance rates, treatment regimens, and steroid use. HIV co-infection has been shown to be a risk factor for death (Hazard Ratio 2.5; 95% CI 1.9-3.4) in Vietnamese adults with TB meningitis,[31] but this remains to be explored systematically in other regions.[32-34] Similarly, neurological disability in adult TB meningitis survivors has not been studied in detail in meta-analyses. In two recent systematic reviews, prevalence of disability in adult TB meningitis survivors varied

between 29% and 50%.[29,30] However neither review provided data on the nature and severity of neurological sequelae in TB meningitis survivors.

We performed a systematic review and meta-analysis to characterize treatment outcomes, namely all-cause mortality and neurological sequelae, for adult TB meningitis across a range of epidemiological settings. We endeavored to perform a definitive review by including the best quality data available and performing a robust quality assessment of the studies included.

Methods

Literature search strategy

This review followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for the reporting of systematic reviews and meta-analyses.[35] A systematic electronic search was conducted using MEDLINE and EMBASE with the aim of identifying all studies reporting treatment outcomes in adult TB meningitis from 1988 to present. This time period corresponds to the WHO recommendation of standard quadruple therapy for the treatment of TB.[26] Controlled and natural language terms identified key search concepts such as: "tuberculosis", "meningitis", "mortality", "complications" and "outcome." Full search strategies are presented in **Appendix A**. Searches were conducted on 9 July 2018.

Study selection

A two-stage sifting process was employed: (1) at title and abstract; and (2) at full text level according to eligibility criteria as detailed below. Sifting was performed in duplicate independently by two reviewers and any unresolved disagreements were

resolved by a third, independent reviewer. Reference and citation checking were conducted for included articles.

Studies were eligible for inclusion if they (i) included adults (aged ≥ 15 years) with confirmed or suspected TB-meningitis; (ii) utilized diagnostic criterion to systematically evaluate patients for TB meningitis; (iii) reported on at least one of the following outcome measures: neurological sequelae, in-hospital mortality, mortality at the end of follow-up (v) employing any of the following study designs: consecutive case series, case control study, cohort study, randomized controlled study, systematic review, or meta-analysis.

The following exclusion criteria were applied: (i) studies with fewer than 10 participants; (ii) studies limited to specific complications or comorbidities (e.g. hydrocephalus, tuberculoma, or surgical intervention); (iii) studies not providing at least a backbone of standard fixed dose combination anti- TB therapy; (iv) studies not specifying treatment given; (v) studies published before 1988; (vi) studies not written in English; (vii) any systematic review superseded by an updated systematic review; (viii) narrative reviews not adding new data or new analysis of data to existing knowledge.

Data extraction and data synthesis

Two authors independently extracted data on study characteristics, recruitment populations, and treatment outcomes from eligible studies using a standardized, piloted electronic data capture database (REDCap, Vanderbilt University, USA). We captured data on geographical region, number of HIV-positive participants, British Medical Research Council (MRC) TB meningitis grade at presentation, treatment regimens utilized, use of corticosteroids, and outcomes reported at specified time points for each

study. Any unresolved disagreements in extraction were resolved by a third, independent reviewer.

We used each study's definition of neurological sequelae as reported in the study. For articles that utilized the modified Rankin Scale or the Barthel index, "disability" was defined as 'any disability that impeded the patient's ability to carry out tasks they once performed'. This is was represented as a score of >2 on the modified Rankin Scale or <80 on the Barthel Index.

For systematic reviews, individual study level data were not extracted or analyzed, only the summary estimates were recorded for comparison, and citation checking was performed to ensure all relevant source manuscripts had been identified.

Data analysis

We used the proportion of all-cause deaths and neurological sequelae within each study to define outcomes of TB meningitis for the meta-analyses. As such, all meta-analyses used random effects models and employed the DerSimonian and Laird method on Freeman-Tukey transformed proportions, which is the established approach for this type of analysis.[36-38] We graphically displayed data in forest plots, which display point estimates of TB meningitis outcomes in each study, with 95% confidence intervals. We generated pooled effect estimates by inverse-variance weighting each individual point estimate such that the estimates with lower variances contributed more to the pooled estimate.[38] The overall pooled estimate for mortality was stratified by follow-up outcome reporting time. Inter-study and sub-group heterogeneity were assessed with the I^2 statistic. All analyses were conducted in Stata version 15.1 (StataCorp, College Station, TX, USA) with the "metaprop" command.[39]

Quality assessment

The 39 articles included in the meta-analysis were assessed for study quality using the Downs and Black tool, a 27-item quality assessment checklist.[40] Each study was scored on a 32-point scale for items that examined quality of reporting, external validity, internal validity (bias and confounding), and study power. Study power was estimated according to sample size methodology. Studies were scored as follows; 0 if no sample size calculation was made or reported in the manuscript (given for observational studies); 3 if a power calculation was done but there were insufficient numbers of patients recruited; 5 if the power calculation was done and sufficiently powered. Systematic reviews meeting the inclusion criteria were not assessed for risk of bias. As treatment outcomes were of interest in these analyses and not treatment or intervention efficacy, we included all studies regardless of quality assessment score.

Results

Search results, studies, and participants included

Our searches yielded 2,562 reports, after removal of duplicates (n=365), 2,197 studies underwent title and abstract screening, and 264 full texts were reviewed (**Figure 2.1**). 39 studies met our eligibility criteria for inclusion and analysis (**Table 2.1**). These 39 studies were published between 1995 and 2018 of which: 10 (26%) were case series, 21 (54%) were cohort studies, and eight (21%) were randomized controlled trials. Studies arose from 18 countries including a range of epidemiological settings; 24 (62%) were from high-TB burden settings and 15 (38%) were from low-TB burden settings.

Figure 2.1. Flow Diagram of Study Selection Process

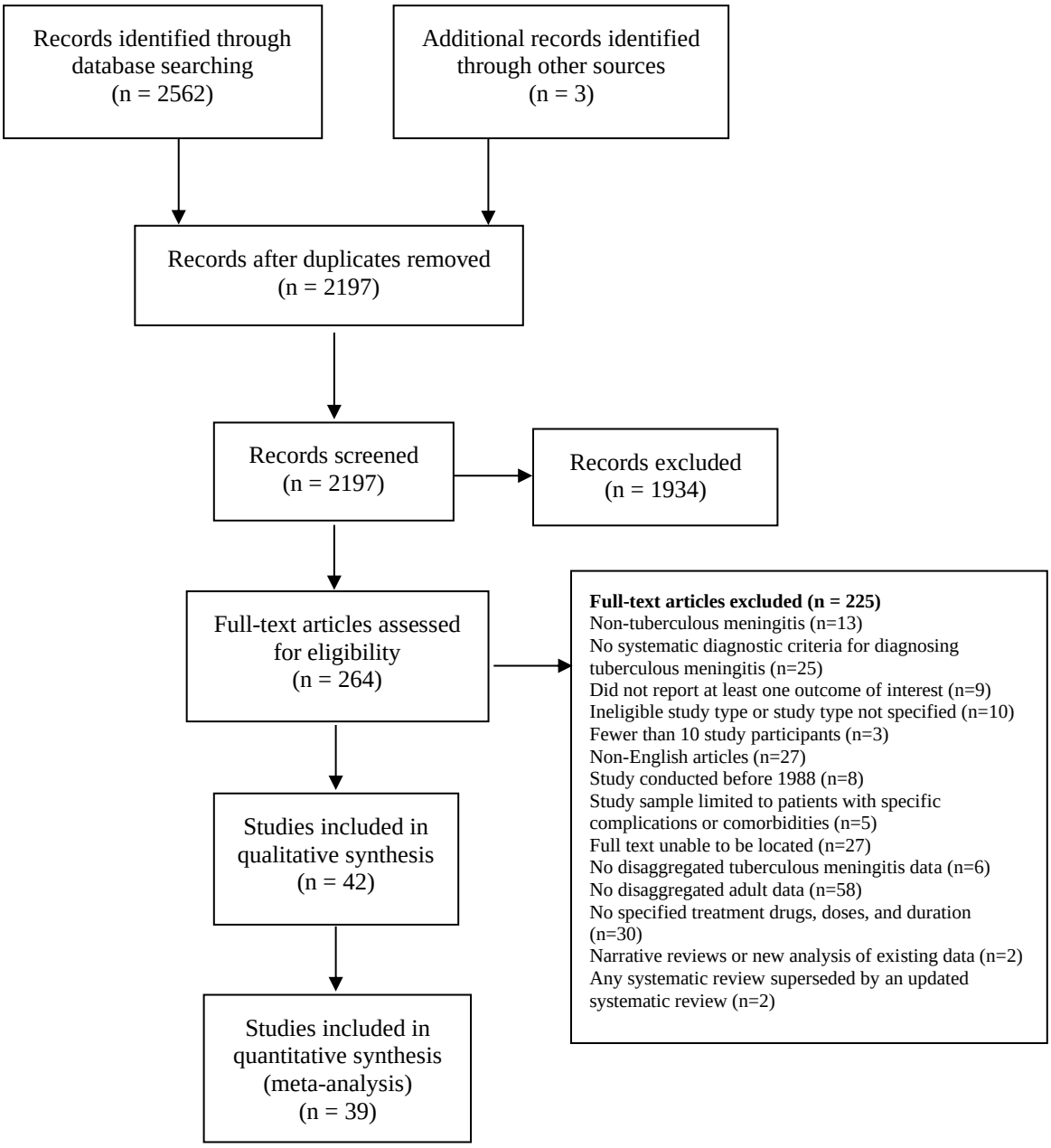


Table 2.1. Characteristics of Studies Meeting Inclusion Criteria

First Author	Year	Study Design	Country	N	Diagnostic Criteria[1]	HIV	Confirmed TBM	Suspected TBM[2]	Antituberculous treatment[3]	Steroids[3]	Outcome(s) and Time point reported
AFRICA											
Luma [41]	2013	Case Series	Cameroon	54	2 a b	54 (100%)	1 (2%)	53 (98%)	2RHZE/6-8RH	All received steroids: unspecified drug(s), dose, & duration	In-hospital mortality
Marais [42]	2011	Cohort	South Africa	120	2 a b c d e f	106 (88%)	47 (39%)	73 (61%)	RHZE	Not specified	In-hospital and 6-month mortality
Thinyane [43]	2015	Case Series	Lesotho	22	2 a b e f	15 (68%)	0 (0%)	22 (100%)	RHZE	Not given	Mortality at the end of follow up
Cresswell [44]	2018	Cohort	Uganda	195	2 a b c d	106 (54%)	74 (38%)	93 (48%)	RHZE	Not specified	In-hospital mortality
Raberahona [45]	2017	Case Series	Madagascar	75	1	3 (4%)	8 (11%)	44 (59%) probable 23 (31%) possible	2RHZE/6RH + S if prior TB (n=2)	Not given	Mortality at 8 months
SOUTH AMERICA											
Gonzalez-Duarte [46]	2011	Cohort	Mexico	64	2 a c f	14 (22%)	44 (69%)	20 (31%)	2RHZE/RH - mean time of therapy was 11.9 ± 7 months	57 (78%) received steroids, unspecified drug(s), dose & duration	Mortality and neurological outcomes at 5 months
Alarcon [47]	2013	Cohort	Ecuador	310	2 a b d e f g h	2 (1%)	140 (45%)	170 (55%)	2RHZ + E or S or quinolone / 10RH (quinolone given to some)	Steroids given to patients with severe disease, unspecified drug(s), dose & duration	Mortality and neurological outcomes at 12 months
ASIA											
Torok [48]	2008	Cohort	Vietnam	58	2 b d e f	58 (100%)	54 (93%)	4 (7%)	3RHZE + S if prior TB/6RH	D (0.3-0.4mg/kg) tapered over 6-8 weeks	Mortality at 9 months
Torok [49]	2011	RCT	Vietnam	253	2 a b d e f	253 (100%)	158 (62%)	95 (38%)	3RHZE + S if prior TB/6RH	D (0.3-0.4mg/kg) tapered over 6-8 weeks	Mortality and neurological outcomes at 9 and 12 months
Heemskerk [31]	2016	RCT	Vietnam	817	1	349 (43%)	407 (50%)	214 (26%) 174 (21%)	2RHZE/6RH + S if prior TB + L in one trial arm	D (0.3-0.4mg/kg) for 6-8 weeks	Mortality at 9 months

First Author	Year	Study Design	Country	N	Diagnostic Criteria[1]	HIV	Confirmed TBM	Suspected TBM[2]	Antituberculous treatment[3]	Steroids[3]	Outcome(s) and Time point reported
Thwaites [50]	2002	Cohort	Vietnam	56	2 a b d	11 (20%)	56 (100%)	0 (0%)	3RHZE/6RHZ if HIV+ 3RHZS/6RHZ if HIV-	Not given	Mortality at 3 months
Thwaites [51]	2004	RCT	Vietnam	545	2 a b d e f	98 (18%)	187 (34%)	358 (66%)	3RHZS/6RHZ 3RHZE/6RHZ if HIV+ or prior history of TB	D (0.3-0.4mg/kg) tapered over 4 weeks, then oral treatment (4mg/day) tapered for 4 weeks	Mortality and neurological outcomes at 9 months
van Laarhoven [33*]	2017	Cohort	Indonesia	608	2 a b c d	93 (15%)	336 (55%)	272 (45%)	RHZE (n=47: high dose R) (n=25: M instead of E)	91% received steroids Drug, dose, and duration not specified	Mortality at 12 months
Singh [52]	2016	Cohort	India	141	1	13 (9%)	54 (38%)	87 (62%)	2RHZS/7HE	D (0.3-0.4mg/kg) tapered over 4 weeks, then oral treatment (4mg/day) tapered for 4 weeks	Neurological outcomes at 9 months
Tai [53]	2016	Cohort	Malaysia	36	1	3 (8%)	23 (64%)	13 (36%)	2RHZE/10RH	Not specified	Neurological outcomes at 3 months
Chen [54]	2014	Cohort	Taiwan	38	2 b d f g	2 (5%)	not reported	not reported	2RHZE/10-16RHE	D (12-16mg) P (60-80mg) tapered 6-8 weeks	Mortality and neurological outcomes at 18 months
Kalita [55+]	2014	RCT	India	60	2 a b c d e f	3 (5%)	24 (40%)	36 (60%)	RHZE	P (0.5 mg/kg/day) for 1 month, tapered over 4 weeks	Mortality and neurological outcomes at 6 months
Sheu [56]	2012	Case Series	Taiwan	91	2 b c d e f g	3 (3%)	not specified	not specified	RHZE +/- S	Either D 12-16mg/day or P 60-80mg/day over 1.5-2 months	In-hospital mortality and neurological outcomes
Wasay [57]	2014	Case Series	Pakistan	404	2 a b d e f g h	1 (0.2%)	35 (9%)	369 (91%)	RHZE + 8% (n=34) received S	unspecified regimen given to all	Mortality and neurological outcomes at 2 months
Chotmongkol [58]	1996	RCT	Thailand	59	2 a	0 (0%)	6 (10%)	53 (90%)	2RHZS/4RH	29 (52%) P 60mg tapered over 5 weeks	Mortality and neurological outcomes at 6 and 18 months

First Author	Year	Study Design	Country	N	Diagnostic Criteria[1]	HIV	Confirmed TBM	Suspected TBM[2]	Antituberculous treatment[3]	Steroids[3]	Outcome(s) and Time point reported
Lu [59]	2001	Cohort	China	36	2 a c d e f	0 (0%)	23 (64%)	13 (36%)	RHZE +/- C and/or S for drug toxicity	Unspecified steroid given to patients with clinical deterioration	Mortality and neurological outcomes at 3 and 6 months
Wang [60]	2002	Cohort	China	41	2 a d f g	0 (0%)	22 (54%)	19 (46%)	RHZE	Unspecified steroid given to 9 patients	Mortality at 6 months
Chotmongkol [61~]	2003	Cohort	Thailand	45	2 a b d	0 (0%)	2 (4%)	42 (93%)	2RHZS/4RH	Not given	Mortality at 6 months
Thwaites [62]	2003	Cohort	Vietnam	21	2 a b d e f g	0 (0%)	15 (71%)	6 (29%)	3RHZS/6RHZ	Not given	Mortality and neurological outcomes at 9 months
Malhotra [63]	2009	RCT	India	91	2 a b e f	0 (0%)	18 (20%)	73 (80%)	2RHZE or S/7RH	D (0.3-0.4mg/kg) tapered over 4 weeks, then oral treatment (4mg/day) tapered for 4 weeks OR MP 5 days OD of either 1 g (weight>50 kg) or 20 mg/kg (<50kg).	Mortality and neurological outcomes at 6 and 18 months
Hsu [64]	2010	Case Series	Taiwan	108	2 a d c e f g h	0 (0%)	46 (43%)	62 (57%)	6RHZ,+ S, C, or L in case of toxicity or side effects	P (minimum 20mg) for >7 days given for 1 to >4 weeks in n=106	Mortality at 9 months
Sharma [65]	2013	Case Series	India	42	2 a e f g	0 (0%)	4 (10%)	38 (90%)	RHZE	Six weeks of steroids, unspecified drug(s) & dose	Mortality and neurological outcomes at 6 months
Sun [66]	2014	Cohort	China	33	2 a d e f h	0 (0%)	7 (21%)	26 (79%)	RHZE +/- PAS + L if in trial arm 2	D 1.5 - 15 mg/d for 1.5-6 weeks	In-hospital neurological outcomes
Kalita [67]	2014	Case Series	India	34	2 a b c d e f h	0 (0%)	34 (34%)	0 (0%)	9RHZE/9RH	P (0.8mg/kg, max 40mg) for 1 month	Mortality and neurological outcomes at 6 months
Imam [68]	2015	Case Series	Qatar	80	2 a b c d e f g h	0 (0%)	35 (44%)	45 (56%)	RHZE + 4% received S, M, and A	D (med 21mg/day) P (med 40mg/day) over 3-9 weeks	Mortality and neurological outcomes at 12 months
Zhang [69]	2016	Cohort	China	401	1	0 (0%)	131 (33%)	202 (50%)	RHZE + L	Not specified	5-year mortality

First Author	Year	Study Design	Country	N	Diagnostic Criteria[1]	HIV	Confirmed TBM	Suspected TBM[2]	Antituberculous treatment[3]	Steroids[3]	Outcome(s) and Time point reported
Kalita [70]	2016	RCT	India	57	2 a b d e f h	0 (0%)	18 (32%)	39 (68%	6RHZE + L in trial arm/12RH for following year	P (0.5 mg/kg/day) for 1 month tapered over 1 month	Mortality and neurological outcomes at 3 and 6 months
Li [71]	2017	Case Series	China	154	1	0 (0%)	18 (12%)	98 (61%) probable 42 (27%) possible	2-4RHZE/6-12RH	D (early treatment), unspecified dose & duration	Mortality and neurological outcomes at 8 months
Mai [72]	2018	RCT	Vietnam	120	1	0 (0%)	92 (77%)	26 (22%)	3RHZES/6RH	D (0.3-0.4mg/kg) tapered over 4 weeks, then oral treatment (4mg/day) tapered for 4 weeks	Mortality and neurological outcomes at 2 and 8 months
EUROPE											
Cagatay [73]	2004	Cohort	Turkey	42	2 a b d e f g h	2 (5%)	10 (24%)	32 (76%)	3-6RHZE	D (8mg) for 4-6 weeks given to patients who were stage II or III	Mortality at 12 months
Doganay [74]	1995	Cohort	Turkey	72	2 a b d f	0 (0%)		72 (100%)	51%: 2RHZS/6RH 49%: various combinations 12-16 months R, H, Z, E, S	P or D 4-6 weeks if MRC stage 3 diseases / signs of raised ICP	Mortality at 2 years
Sutlas [75]	2003	Cohort	Turkey	61	2 b d e f g h	0 (0%)	19 (31%)	42 (69%)	1RHZES/2-3RHZE/4-9RHZ (if no tuberculoma present)/10-12RH	P (1mg/kg/day) for 1 month, tapered for 4 months	Mortality at 12 months
Sengoz [76]	2008	Cohort	Turkey	121	2 a b d e f g h	0 (0%)	52 (43%)	69 (57%)	2RHZ + E or S/7-10RH	2D (16 mg/day) for those with neurological deficits	Mortality at the end of follow up
Miftode [77]	2015	Cohort	Romania	127	1	0 (0%)	25 (20%)	35 (28%) probable 70 (55%) possible	2-3RHZE/7-9RH	All received: unspecified drug, dose, & duration	In-hospital mortality and neurological outcomes

[1] Diagnostic Criteria Legend;
1= Uniform case definition
2= Other criteria used to diagnose and categorize patients including: a=suggestive CSF picture, b=microscopy, c=Xpert / PCR, d=culture, f=evidence of extra-neural, TB, g=response to treatment, h=other (history of TB or contact with a TB-infected individual, positive mantoux reaction, IGM AB in the CSF, biopsy, etc.)

[2] Some participants were considered 'suspected' as well as 'confirmed' TBM

[3] TB treatment (given to all unless specified otherwise): Number of months placed in front of regimen code: R=rifampicin, H=isoniazid, Z=pyrazinamide, E=ethambutol, S=streptomycin, L=levofloxacin, M=moxifloxacin, C=ciprofloxacin, A=amikacin, PAS=paraaminosalacylic acid, P=prednisolone, D=dexamethasone, MP=methylprenisolone. Where no duration of antituberculosis therapy or steroids is stated it means it was not clearly specified in the paper.

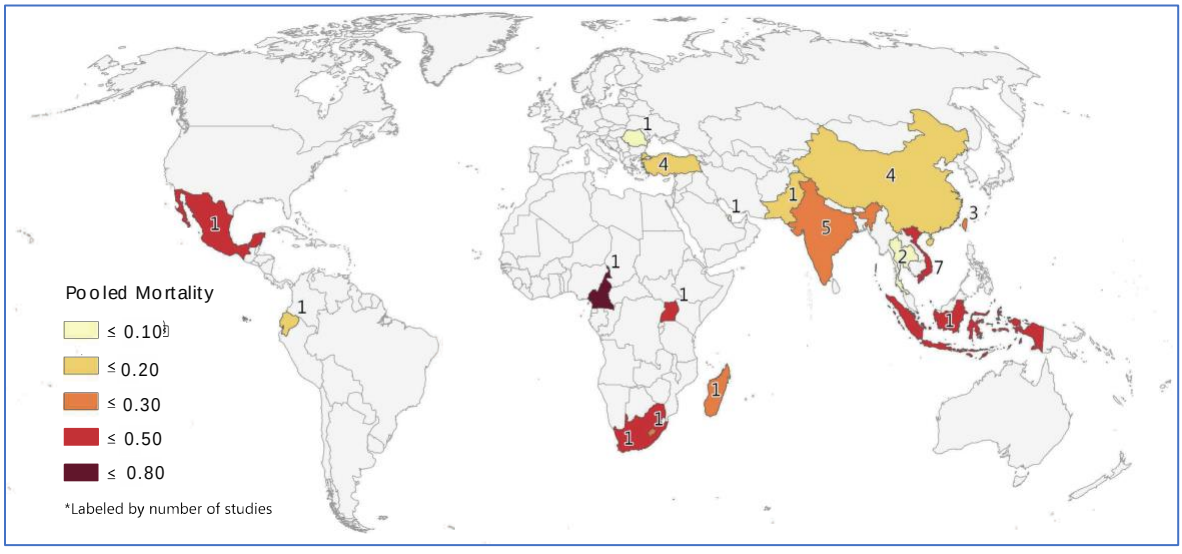
[*] van Laarhoven et al. includes some data from 3 clinical trials in Indonesia (Ruslami R, Lancet Infect Dis, 2013, Yunivita V, Int J Intimicrob, 2016. Dian S, Antimicrob Agents Chemother. 2018). The primary studies were excluded from the review to avoid duplication of data.

[+] Only included participants that were treated with RHZE

[~]Treatment information was taken from Chotmongkol 1996 as they were from the same authors, hospital, and decade.

A total of 26 (67%) studies were conducted in Asia, and five (13%), five (13%), and two (5%) in Europe, Africa, and the Americas, respectively (**Figure 2.2**). Study quality scores ranged from eight to 32, with a score of 32 indicating the highest quality. Median quality score for included articles was 18 (IQR; 15-20). Our meta-analysis includes reported treatment outcomes for 5,752 adults with TB meningitis. Participant age ranged from 15 to 88 years. Seven studies included 1,078 HIV-positive patients: 302 (28%) from Africa, and 776 (72%) from Asia. MRC TB meningitis grade was reported in 29 studies, in which 28% (1354/4761) of participants presented with MRC grade I disease, 48% (2302/4761) with grade II, and 20% (967/4761) with grade III. A total of 37 studies (n=5,623 participants) reported the classification or uniform case definition of enrolled participants. Of those, 40% (2,243/5,623) were microbiologically-confirmed TB meningitis, 49% (2,741/5,623) were suspected TB meningitis, the latter of which included 21% (1,013/5,623) with probable TB meningitis and 12% (663/5,623) with possible TB meningitis according to the uniform case definition.[10]

Figure 2.2. Tuberculous Meningitis Mortality by Country



The most common treatment regimen was standard four-drug therapy of rifampicin, isoniazid, pyrazinamide, and ethambutol (RHZE) with no additional anti-TB drugs (n=17 studies). Seven studies used streptomycin in addition or in replacement for ethambutol (**Table 2.1**). Median treatment duration was nine months (IQR; 9-12 months). Corticosteroids were given to all patients in 19 studies, and to some participants in 10 studies (**Table 2.1**)**.** Treatment outcomes by corticosteroid use was examined in a meta-analysis with included studies, but this was not the aim nor design of our meta-analysis and a significant amount of heterogeneity in mortality between studies was unexplained (**Appendix B**). A Cochrane meta-analysis on corticosteroid use in TB meningitis was published in 2016.[78]

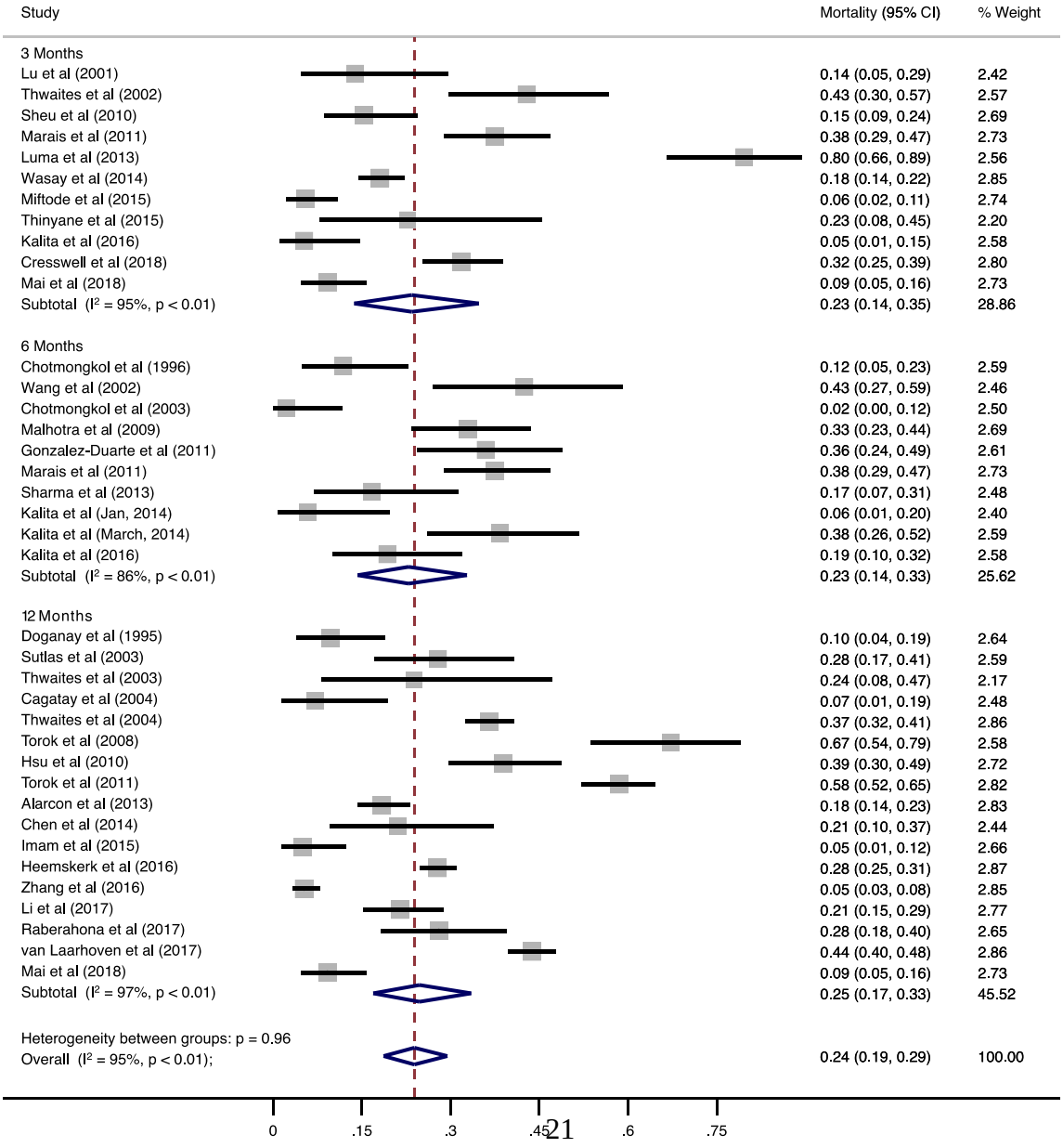
Mortality assessment and outcomes

A wide range of mortality endpoints were reported: 15% (6/39) studies reported one-month mortality, 5% (2/39) studies reported two-month mortality, 8% (3/39) studies reported three-month mortality, 18% (7/39) studies reported six-month mortality, 13% (5/39) studies reported 12-month mortality, and 2% (1/39) reported five-year mortality. Other reported outcomes included in-hospital mortality (n=6 studies) and median-time to death (n=4 studies). In the six studies which reported on 'in-hospital mortality', only one study reported on the length of hospitalization which ranged from 4-10 days until death or discharge. Five studies did not define the 'in-hospital mortality' in terms of time frame.

To investigate time-specific mortality, articles were grouped by follow-up outcome reporting time point. Articles that reported outcomes less than or equal to three months were included in the three-month reporting category to summarize 'early'

mortality. Articles that reported outcomes greater than three months to six months were included in the six-month reporting category. Articles that reported outcomes greater than six months were included in the 12-month reporting category. Of articles reporting outcomes at three, six, and 12 months, pooled mortality was 23% (95% CI; 14-35%), 23% (95% CI; 14-33%), and 25% (95% CI; 17-33%), respectively (**Figure 2.3**). There was significant heterogeneity (I^2 = 95%; p<0·01) for all outcome reporting timepoints.

Figure 2.3. Tuberculous Meningitis Mortality by Outcome Reporting Timepoint

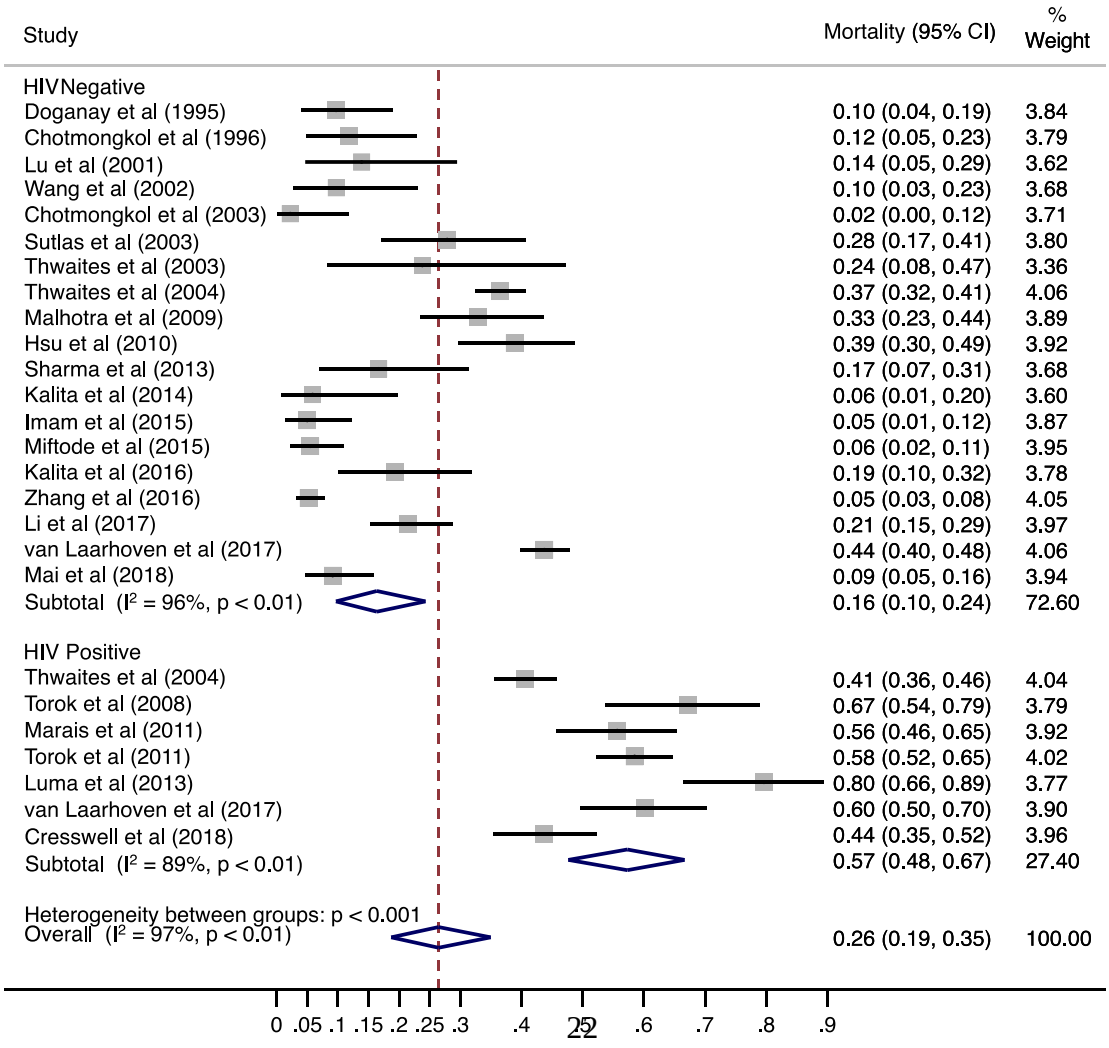


There was no marked heterogeneity in mortality between outcome reporting timepoints (p=0·60), but it was included in the pooled analysis resulting in a pooled mortality of 24% (95% CI; 19-29%).

Mortality endpoints by HIV status

Seven studies reported mortality for HIV-positive adults. For HIV positive adults, pooled mortality was 57% (95% CI; 48-67%), compared with 16% (95% CI; 10-24%) in HIV-negative adults (**Figure 2.4**). HIV status explained a significant amount of the observed heterogeneity in TB meningitis mortality (p< 0·01).

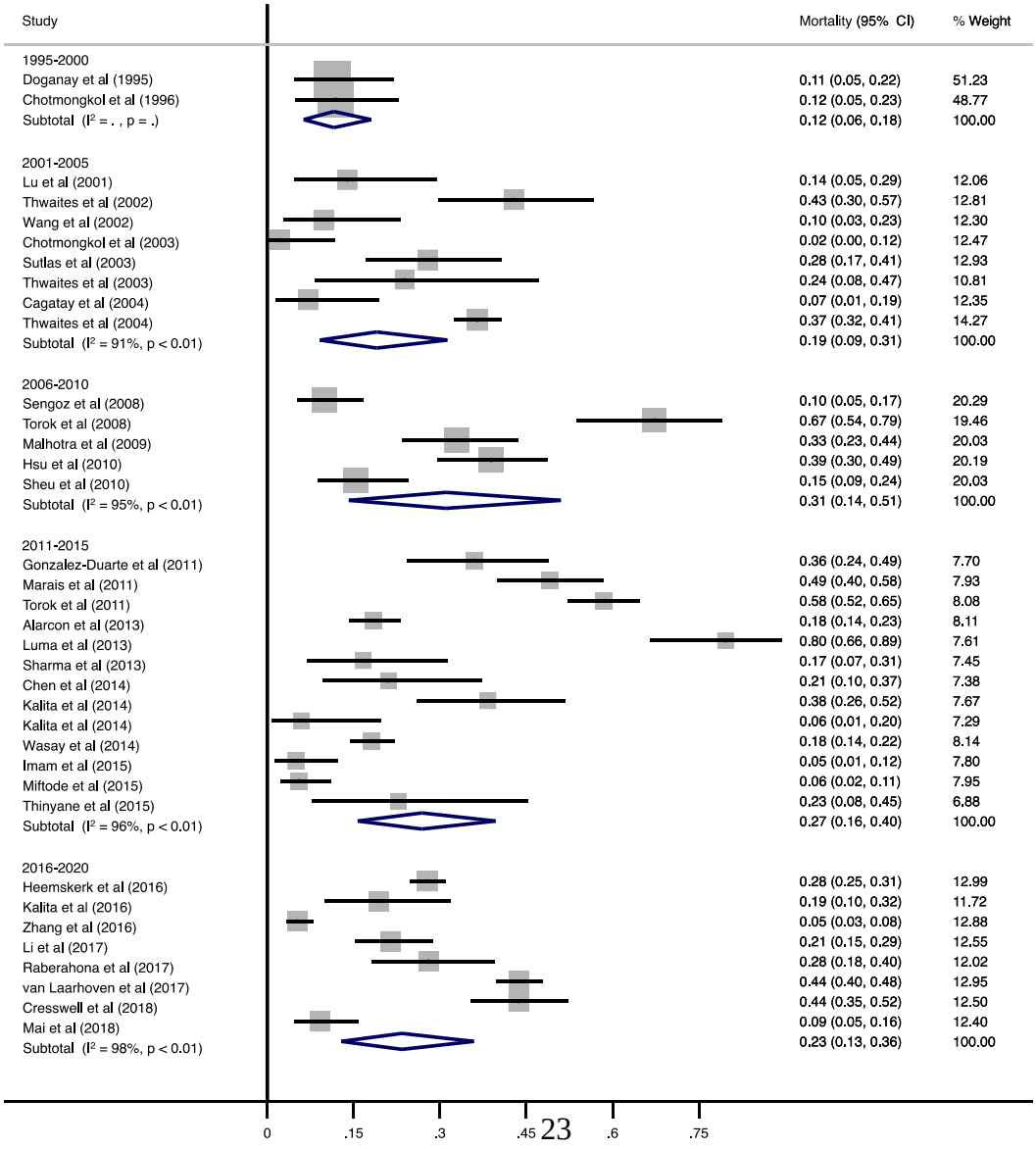
Figure 2.4. Tuberculous Meningitis Mortality by HIV Status



Mortality endpoints by geographical region

Most studies reporting on TB meningitis mortality were conducted in India and the Asian continent (n=27; 70%) where pooled mortality ranged from 2-67% (**Figure 2.2**). The countries reporting the highest TB meningitis mortality were located in sub-Saharan Africa where mortality ranged from 23-80%. Continent (Africa vs. Asia) explained a significant amount of the observed heterogeneity in TB meningitis mortality (p=0.02).

Figure 2.5. Tuberculous Meningitis Mortality by Year Published



Temporal variation in mortality endpoints

To investigate changes in TB meningitis treatment outcomes over time, we conducted a temporal analysis in which individual studies were allocated to one of five time periods and stratified analyses conducted. Time periods were sub-divided into five-year windows from 1995 onwards, and pooled mortality analyzed within each time window. Highest pooled mortality was 31% (95% CI; 14-51%) in articles published from 2006-2010, though there was no significant variation by time window (**Figure 2.5**). In earlier time periods, the heterogeneity in survival was greatest and heterogeneity appears to have reduced in the more recent time periods.

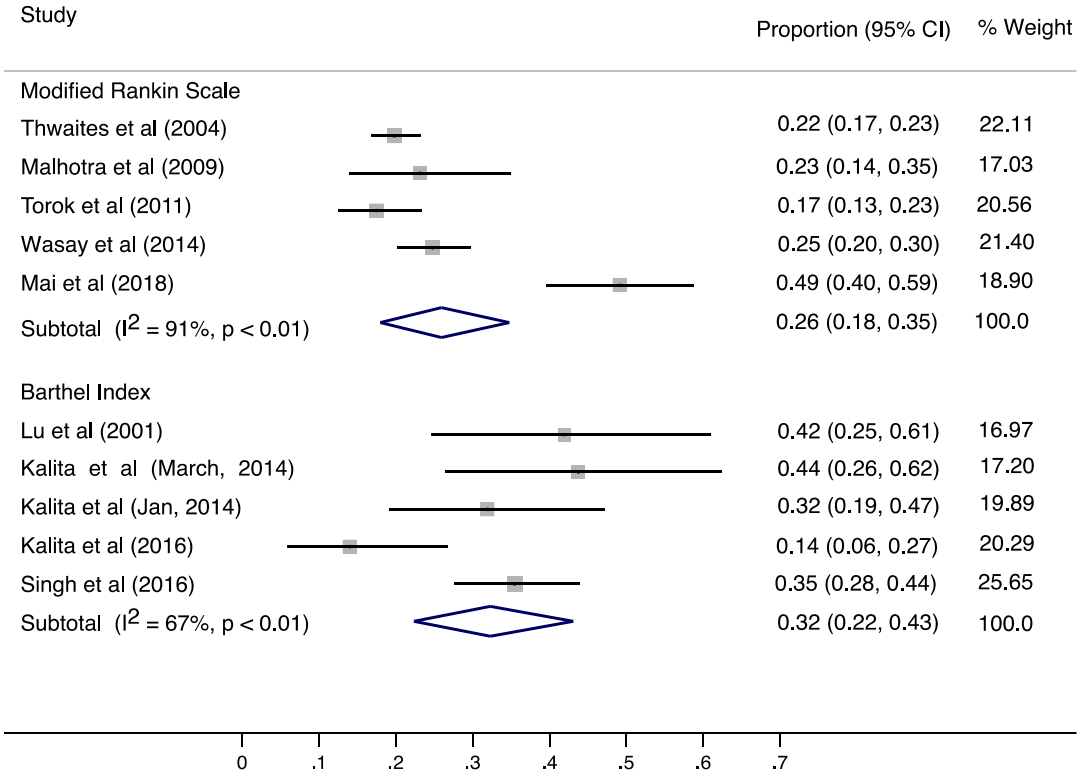
Neurological disability

Functional outcomes among survivors was a pre-specified endpoint in 24 studies; 10 studies reported on functional outcomes using the modified Rankin Scale score (n=6) or the Barthel index (n=5), and 10 studies reported on neurocognitive disability without using a specified scale or measurement tool, and five studies reported using "clinical assessments".

The timing and method of neurological assessments varied between studies; the most commonly used outcome assessment being physical disability conducted at the end of follow up. In this analysis, participants were considered disabled if there was any indication of functional disability as reported by the modified Rankin Scale or Barthel Index. Of the studies utilizing the modified Rankin Scale, the pooled proportion of patients experiencing some level of physical disability was 26% (95% CI; 18-35%) with considerable heterogeneity (**Figure 2.6**). Of the studies using the Barthel Index the

proportion of patients experiencing some level of physical disability was 32% (95% CI; 22-43%) with only moderate heterogeneity.

Figure 2.6. Physical Disability by Modified Rankin Scale and Barthel Index



Discussion

In this rigorous systemic review and meta-analysis, we reviewed treatment outcomes for over six thousand adults with TB meningitis, and our data clearly demonstrate that the mortality and neurological sequelae associated with TB meningitis remains unacceptably high. Although there was significant heterogeneity between studies (I^2 >95%), overall risk of death was 23% at three months, and 25% at 12 months. In patients that did survive, neurological sequelae were common, affecting nearly one third of all patients. Furthermore, our temporal analysis of treatment outcomes indicate that prognosis has improved little over time. Our results are in concordance with two recently

published systematic reviews which reported overall morality associated with adult TB meningitis to be 23% and 25%, and risk of neurological sequelae to be 29% and 50%, respectively. Our study expands on the current literature through sub-group meta-analyses to evaluate differential treatment outcomes by HIV status and geographical region.

We have demonstrated that patients with HIV-associated TB meningitis have three-fold higher mortality compared to HIV-negative cohorts; mortality in HIV-negative cohorts ranged between 10-24% compared to 48-67% in HIV-positive cohorts ($p< 0.01$). Pathogenesis research is urgently needed to investigate the disproportionate mortality associated with HIV co-infection in TB meningitis, and to identify potential interventions or preventative measures.

Secondly, our data demonstrate that despite adoption of standardized treatment regimens for TB meningitis, considerable global disparities in treatment outcomes exist. Pathogenesis work has shown that even within a Vietnamese population a single genetic polymorphism significantly impacts on corticosteroid responsiveness and survival from TBM.[79] The extent of the heterogeneity observed in this meta-analysis raises the possibility that genetic or other latent factors may contribute to outcome and the current one-size-fits all approach to treatment may be effective in some individuals/populations and less effective in others. Our sub-group meta-analyses indicate that patients in the African continent have a higher mortality compared to all other continents. This may in part be explained by the higher co-prevalence of HIV. However, given the considerable resource limitations including a lack of intensive care facilities typical of many settings in sub-Saharan Africa, it is likely that the management of commonly encountered

complications of TB meningitis including hyponatremia, raised intracranial pressure, hydrocephalus, stroke, and nosocomial infections are suboptimal. Further research is needed to determine the attributable mortality due to a lack of supportive or critical care in sub-Saharan Africa. Our systematic literature review highlights the historical paucity of clinical studies published from this continent. In order to address the devastatingly poor outcomes from HIV-associated meningitis, particularly for those in sub-Saharan Africa, we need to design, fund and deliver more clinical research.

Our meta-analyses of follow-up time-specific mortality at three, six, and twelve months, highlight that over 90% of TB meningitis deaths occur in the first three months. This may justify that three-month mortality is a reasonable RCT endpoint, potentially making study trial follow-up shorter and cheaper, and therefore accelerating research outputs. However, the considerable heterogeneity found in these analyses as well as inconsistencies in reporting outcomes, indicates that further evidence is needed to justify a three-month clinical trial endpoint. Clinical studies to identify drivers of early mortality in TB meningitis may inform the design of treatment intensification strategies and other adjunctive interventions.

Concerningly, our results demonstrate that minimal improvements in survival have been made over time. There are a number of temporal factors which may have affected outcomes in certain time periods including the height of the HIV epidemic in the 1990-2005 period, ART rollout in the 1995 to 2010 windows, the increasing availability of more rapid diagnostics in the form of the Xpert MTB/Rif assay in 2010 to 2020 windows facilitating the diagnosis of TB meningitis where it was previously

unconfirmed, and lastly gradually increasing rates of anti-TB drug resistance worldwide. Reporting bias, which may have varied over time, must also be considered.

Our analysis has several limitations. Firstly, although we only included studies which employed a pre-specified diagnostic criterion for TB meningitis, there was considerable variation in the quality of diagnostic criteria used, and diagnostics have changed over time. We chose not to restrict diagnostic criteria to microbiologically confirmed TB meningitis, because doing so would have restricted our meta-analysis to 40% (n=2,243) of adults, and furthermore we wanted our results to be generalizable to real world clinical settings where confirmation rates are often only moderate. We do however recognize that misclassification of undifferentiated meningitis cases as TB meningitis is common, especially when left to physician discretion; as may have been the case in some of the patients included in our meta-analysis and therefore this would undermine the accuracy of our outcome estimates. Secondly, in the spirit of generalizability we chose to include case-series, which are primarily descriptive and not wholly representative of the populations they are drawn from. Although this may have posed some unmeasurable bias, we believe that this would not have substantially impacted our results since mortality and neurological sequelae, our outcomes of interest, would not have measured differently or changed based on study design. Thirdly, the specific anti-TB regimen utilized and drug resistance rates within the cohorts was inconsistently reported in studies therefore we were unable to conduct stratified meta-analyses based on drug resistance patterns. The International Tuberculous Meningitis Research Consortium paper on standardized methods for enhanced quality and comparability of TB meningitis studies, specify that it is essential to document the dose,

route of administration, and duration of all anti-TB drugs used in TB meningitis studies.[80] There remain several outstanding questions concerning the optimal treatment of TB meningitis, and therefore to facilitate cross study comparisons and interrogate differences in study outcomes basic information about the treatment provided is essential.

Finally, there was a considerable lack of standardization of reporting on treatment outcomes. This was particularly marked with respect to reporting of neurological sequelae; firstly, neurological sequalae were rarely reported (only 10/39 (26%) studies including any data on neurological sequalae), the tools used were inconsistent (nine tools in total) and the time-points for assessment were rarely reported. This inconsistent reporting hampered comparison of data across studies. Given the importance of neurological disability in TB meningitis and the importance of developing a standardized evidence base against which to assess new treatments, the International Tuberculous Meningitis Research Consortium recommend that the modified Rankin Score should be used as the first line tool, which should be recorded at 12 months from antituberculosis treatment initiation in all adults.[80] We support this recommendation, and in addition would suggest that mortality be routinely reported on at three, six, and 12 months if possible, to improve study comparability.

The strengths of this work include its size, with 39 individual studies included studies from Asia, Africa, Europe and the Americas making our estimates broadly generalizable to a range of settings. Our systematic review is larger than two previously published systematic reviews of adult TB meningitis.[29,30] In comparison to Wen et al,[29] we decided to include randomized control trials in our systematic review which enable us to include the highest quality of trial evidence, and we also reported drug resistance rates

within each included study. In comparison to Wang et al,[30] we ascertained variation in treatment outcomes geographically, and reported on the nature and severity of reported neurological sequelae. Overall, we assessed a wide range of co-variates to investigate the heterogeneity in treatment outcomes observed. To our knowledge, this is the most extensive critical appraisal of TB meningitis outcomes to date.

In conclusion, adult TB meningitis is associated with considerable neurological morbidity and mortality and remains a major challenge in TB endemic regions. The worst outcomes are observed by those with HIV co-infection in sub-Saharan Africa where risk of death is three-fold higher. Our study was limited by suboptimal reporting on diagnostic criteria utilized, drug resistance rates, details of treatment regimens used, as well highly variable outcome reporting. Adoption of standardized reporting systems across TB meningitis studies would not only facilitate across study comparisons, but overall would also improve the quality of research outputs and support collaborative research across centers with an aim of improving TB meningitis outcomes globally.

CHAPTER 3. MANUSCRIPT 2: DEVELOPMENT AND VALIDATION OF A CLINICAL PREDICTION TOOL FOR TUBERCULOUS MENINGITIS

Introduction

Tuberculosis remains a major global health problem, and tuberculous (TB) meningitis is the most lethal and disabling form, representing more than 100,000 new cases each year.[81] Current diagnostic evaluations are lengthy, inaccessible, or insensitive leading to delayed diagnosis and treatment – significant risk factors for poor outcomes.[81] Ziehl-Neelsen staining of CSF has low sensitivity in most settings, and mycobacterial culture is typically too slow to inform treatment decisions. While recently introduced nucleic acid amplification tests (NAATs) have the potential to speed up diagnosis, variable sensitivity in clinical settings means that negative tests are often insufficient to justify withholding treatment.[9] Furthermore, there is a disproportionate burden of TB meningitis in low-resource settings, where access to NAATs is limited. An additional barrier to increased knowledge regarding the true prevalence, incidence, and mortality of TB meningitis as well as the evaluation of novel diagnostics is the lack of an agreed reference standard that is 100% accurate in all settings.[9]

One approach to improving rapid diagnosis of TB meningitis is to develop and validate multivariable prediction models (MPM) for clinical use. At least 10 MPM for TB meningitis have been developed, which tend to perform well in internal validation but poorly when externally validated in different settings or populations. The primary contributing factor of heterogeneous model performance across different settings and populations is case mix variation, which refers to the distribution of important predictor variables such as HIV status, age, and the prevalence of TB meningitis. Case mix variation across different settings or populations can lead to genuine differences in the

performance of a prediction model, even when the true predictor effects are consistent (that is, when the effect of a particular predictor on outcome risk is the same regardless of the study population).[25] Prior MPMs for the diagnosis of TB meningitis were all developed from a single study or population, and therefore have not adequately accounted for case-mix variation, compromising external validity and clinical utility.

Furthermore, most approaches to MPM development utilized logistic regression with stepwise (backwards or forwards) selection of variables into the model, which were retained if they significantly predicted TB meningitis diagnosis (determined a priori with a set p-value threshold). This methodological approach has many limitations including overfitting and the inability to model non-linear associations, including interactions, without pre-specifications.[82-84] Using this approach does not adequately model case-mix variation, which can also lead to overfitting.[83]

To overcome these limitations, we collected individual participant data (IPD) from multiple studies in a variety of geographical locations and evaluated alternative modeling approaches. IPD is preferred to aggregate data meta-analysis as multiple individual level factors related to the disease can be examined in combination.[85] The coalition and synthesis of IPD offers a novel and natural opportunity to overcome the challenge of external validation of previously published MPMs. Recent studies have shown how big data can be used by researchers to examine heterogeneity and improve the predictive performance of a model across different populations, settings, and subgroups.[86-88] Therefore, in addition to logistic regression we also analyzed the data using machine learning techniques, classification and regression tree (CART) and

random forest models. Our findings are reported in accordance with the transparent reporting of a MPM for individual prognosis or diagnosis (TRIPOD) statement.[89]

Methods:

Literature Search Strategy

This review was carried out according to the Preferred Reporting Items for Systematic review and Meta-Analysis of IPD (PRISMA) guidelines.[90] Our protocol is available on Wellcome Open Research (https://doi.org/10.12688/wellcomeopenres.15056.2). A systematic electronic search was conducted using MEDLINE and EMBASE with the aim of identifying all studies reporting treatment outcomes in adult tuberculous meningitis from 1990 to present. This time period corresponds to the WHO recommendation of standard quadruple therapy for the treatment of tuberculosis.[26] Controlled and natural language terms identified key search concepts such as: "tuberculosis", "meningitis", "mortality", "complications" and "outcome." Full search strategies are presented in **Appendix C**. Searches were conducted on 26 September 2018.

Study Selection

The following exclusion criteria were applied; (i) studies published before 1990; (ii) case-control studies and case-series of patients with confirmed TBM (iii) studies not written in English; (iv) studies where IPD are not available; (v) mathematical modeling studies; (vi) any systematic review superseded by an updated systematic review; (vii) studies which collected insufficient data on lab and clinical variables known to predict TB meningitis (viii) studies of participants less than 5 years old.

Studies were eligible for inclusion if they both (i) employed any of the following study designs: cross-sectional study, cohort study, randomized controlled study; (ii) systematically evaluated a sample of patients with suspected TB meningitis with at least one of, Ziehl–Neelsen stain, commercial NAAT for mycobacterium tuberculosis, or mycobacterial culture of CSF.

A two-stage screening process was employed: title and abstracts were screened for eligibility by two researchers; and those considered potentially eligible underwent full review by one researcher.

Risk of bias of included studies was assessed by two independent reviewers using QUADAS-2,[91] discrepancies were resolved by a third reviewer.

Data Acquisition and Synthesis

Corresponding authors of studies identified as eligible after full text review were contacted with a request to provide anonymized IPD. A data sharing agreement was signed before eligible authors uploaded the IPD file to an encrypted and secure cloud storage repository. Uploaded IPD files were harmonized, units were standardized, and data were synthesized in R studio. Specific IPD variables requested are listed in **Table 3.1** and were chosen to include as much information about the participants as possible. Of these variables, we identified 11 target predictors based on prior studies (**Table 1.2**): symptom duration, cerebral spinal fluid (CSF) white blood cell (WBC) count, CSF WBC differential, CSF glucose, CSF protein, blood glucose, blood WBC count, HIV status, age, and biological sex. Subjects who were missing more than 50% of target predictors were excluded. Datasets were excluded if there was a clear pattern of missingness among the target predictors that was based on diagnosis, age, sex, or some other participant

characteristic. IPD that were provided from the same research group were analyzed as a single dataset.

Table 3.1. Individual Patient Data Requested from Authors and Included in Model Development

Clinical Data at Presentation	Blood Results	CSF Results
• Age* • Sex* • Presence of seizures • Presence of neck stiffness • Duration of symptoms* • Focal neurological deficit (including cranial nerve palsy) • Temperature • Glasgow Coma Scale (GCS)	• HIV status* • White Blood cell count • CD4 count, if HIV-positive • Glucose*	• Appearance • WBC Count* • Total or % neutrophils* • Total or % lymphocytes* • Protein* • Glucose* • Gram stain • Adenosine deaminase activity • Bacterial culture • India ink stain • Cryptococcal antigen or culture • Microscopy for acid-fast bacilli • Mycobacterial culture • NAAT for Mycobacterium tuberculosis • NAAT for any virus • Syphilis serology
Urine/Sputum/Other Laboratory Results	**Radiological Results**	**Outcomes**
• Urine LAM • Microscopy for acid-fast bacilli • Mycobacterial culture • NAAT for Mycobacterium tuberculosis	• Chest X-ray • Abdominal ultrasound scan • CT brain • MRI brain	• Outcome (Discharged or Died) • Date of Outcome

*Target predictors
LAM, lipoarabinomannan; NAAT, nucleic acid amplification test

Multiple Imputation

Blood glucose was the variable with the most missingness in every dataset. We performed single imputation of the median value in each dataset for missing blood glucose. For the other target predictors, the MICE package in R studio was used to impute missing values within each dataset prior to merging. Missing data within datasets

were assumed missing at random. A total of 50 imputations were used per missing variable. The fraction of missing information (FMI) statistic was obtained in the modeling step to assess efficiency gains.[92]

Data Analysis

We summarized IPD from each of the studies using medians and proportions for continuous and dichotomous data, respectively. We defined 'confirmed' TB meningitis as having any of the following positive tests in the CSF: Xpert MTB/RIF (Cepheid, Sunnyvale, CA), Xpert Ultra MTB/RIF, other PCR, culture, acid fast bacilli (AFB), or 'definite' TB meningitis classification per the Uniform TBM case definition.[10] We defined 'probable' TB meningitis as having no alternate diagnoses and any CT, MRI, or X-ray suggestive of TB meningitis or a 'probable' TB meningitis case classification per the Uniform TB meningitis case definition.[10] In these analyses, participants that fit the criteria for confirmed or probable TB meningitis were considered a TB meningitis case.

Three algorithm development strategies were employed to predict a binary outcome: TB meningitis cases vs. non-TB meningitis. First, an IPD meta-analysis using a logistic regression model with stratified intercepts for each country was fitted with the selected 11 target predictors.[87] We employed a backwards stepwise method for predictor selection using a p-value threshold of 0.1. We also fitted a logistic regression model without stratified intercepts. Next, we developed CART and random forest models with machine learning methods with the same 11 target predictors as well as a variable for country.

All models were internally validated using an k-fold internal-external cross-validation (IECV) approach, which is a multiple validation approach that accounts for

multiple datasets by rotating which are used toward model development and validation.[87] We amalgamated IPD from studies that originated from the same working group into a single IPD dataset. During the IECV process, each IPD dataset was excluded from the available set, and the remainder was used to develop the MPMs; the excluded dataset was then used to validate the model externally. This process was repeated with each dataset being omitted in turn, allowing the consistency and performance of the developed MPM to be examined across multiple datasets. We measured performance in each study (fold) using the calibration ratio of predicted (expected) to observed outcomes (denoted by E/O), calibration plots (intercept and slope), the area under the receiver operating characteristic (ROC) curve, and the Brier Score.[87,93,94] Good model performance is reflected by the E/O ratio, calibration slope, and ROC values being close to one. We summarized overall MPM internal validity by averaging the values of ROC and amalgamating calibration across the folds in addition to calculating the Brier score. The Brier score is a measure of the accuracy of probabilistic predictions where a value close to 0 indicates perfect accuracy.

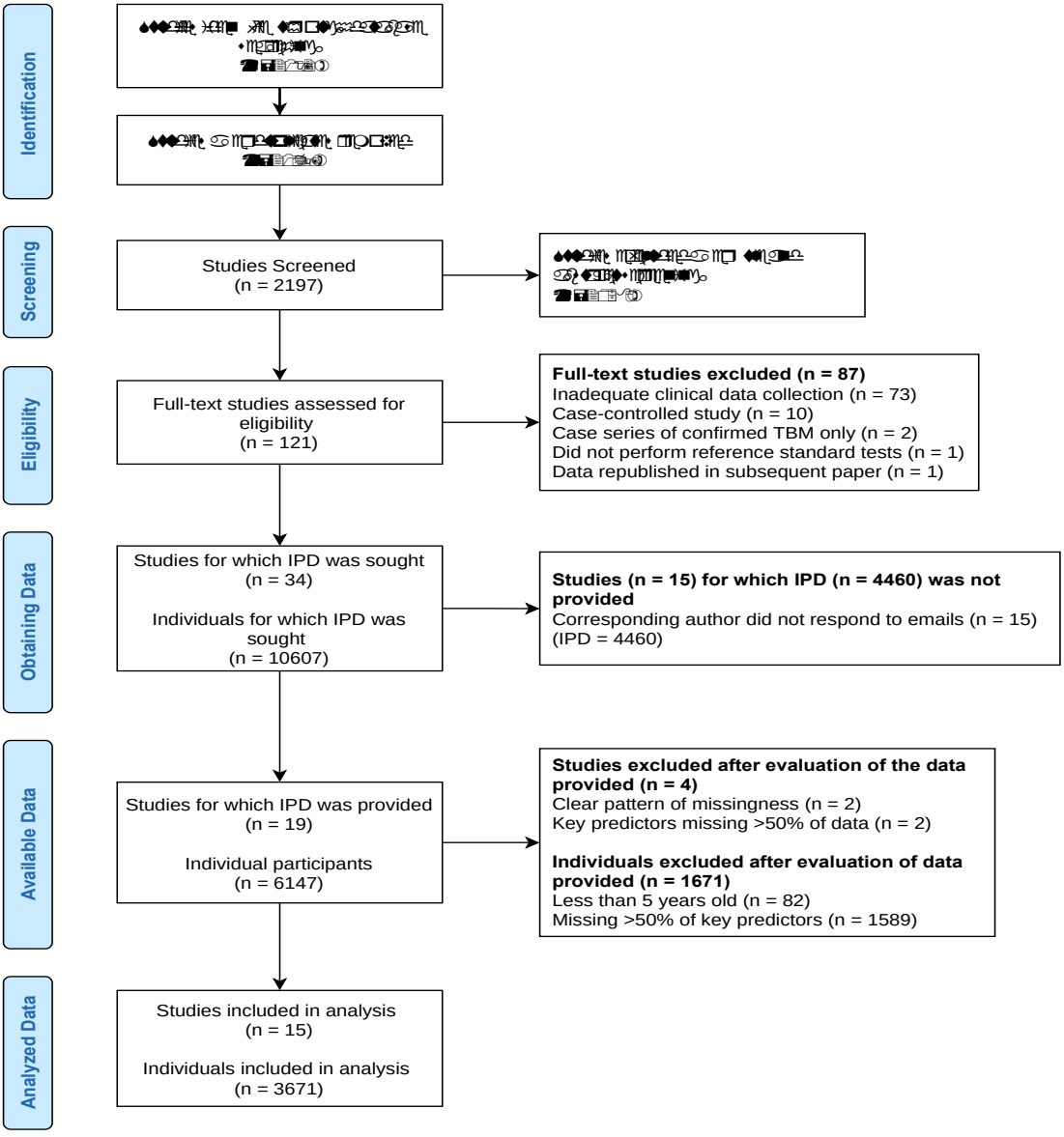
Additionally, we calculated sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR), false negative rate (FNR), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and proportion correctly classified (PCC) in each IPD dataset fold along different prediction thresholds. All analyses were conducted in R studio.

Results

Search results, studies, and participants included

After removal of duplicates, our searches yielded 2179 reports that underwent title and abstract screening, and 121 full texts were reviewed (**Figure 3.1**). 34 studies met our eligibility criteria for inclusion, and we acquired IPD from 19 studies (18 datasets) with a total of 6,147 individual participants. Four datasets (N=796 individual participants) were excluded after evaluation of missingness revealed either missingness based on diagnosis

Figure 3.1. PRISMA IPD Flow Diagram of Study Selection Process



or target predictors were missing >50% of their data. An additional 82 participants less than five years old and 1,589 participants missing >50% of key predictors were excluded.

The final analysis dataset included 3,671 individual participants from 15 different studies (**Table 3.2**). Four studies originated in Brazil[95-98] (N= 101, 321, 289, and 92), three from Vietnam[99-101] (N=160, 303, and 204), two from South Africa (N=93 and 36) [102,103], and one each from Uganda[104] (N=611) Botswana[105] (N=138), Indonesia[33] (N=761), Morocco[18] (N=414), Peru[106] (N=37), and Romania[107] (N=111). The studies conducted by Nhu,[99] Heemskerk,[100] and Donovan[101] in Vietnam, and the studies conducted by Boulware[103] and Rhein[104] in Uganda originated from the same working groups. Most were cohort studies (n=9) or cross-sectional studies (n=4), and two were randomized controlled trials. The risk of bias assessment is summarized in **Appendix D**. No study showed high risk of bias.

A total of 1148 (31%) participants met the case definition for confirmed TB meningitis and 104 (3%) met the case definition for probable TB meningitis. Of the non-TB meningitis cases, 13% had cryptococcal, 6% had bacterial, and 3% had viral meningitis. Most participants had an unknown diagnosis (**Figure 3.2**). The age range of participants was between 5 to 90 years old and was statistically significantly different between TB and non-TB meningitis groups (**Table 3.3**). Among datasets that disclosed biological sex or gender, 58% (n=1746) of participants were men. There was no difference in biological sex or gender between TB and non-TB meningitis groups. With the exception of the dataset provided by Dendane et al.[18], at least 10% of participants were HIV-positive in each study. The final analysis dataset included 1644 (45%) HIV

positive individuals. There was a higher proportion of HIV-positive individuals in the non-TB meningitis group compared to the TB meningitis group ($p<0.001$).

Table 3.2. Characteristics of Studies Included in Analysis Dataset

Author/Owner	Year	Country	TB Burden	Study Design*	N	Age (range)	Men (%)	HIV (%)	Probable Cases (%)	Confirmed Cases (%)
Anselmo[95]	2017	Brazil	High	Cross-Sectional	289	43 (6-84)	163 (56)	142 (49)	10 (3.5)	39 (13)
Gualberto[96]	2017	Brazil	High	Cohort	92	37 (8-64)	65 (71)	92 (100)	6 (6.5)	8 (8.7)
Azevedo[97]	2018	Brazil	High	Cohort	101	40 (17-73)	62 (61)	101 (100)	0 (0)	12 (12)
de Almeida[98]	2019	Brazil	High	Cross-Sectional	321	40 (5-86)	188 (59)	177 (55)	13 (4.0)	13 (4.0)
Nhu[99]	2014	Vietnam	High	Cross-Sectional	160	NA	NA	64 (40)	24 (15)	132 (83)
Heemskerk[100]	2018	Vietnam	High	Cohort	303	NA	NA	38 (13)	0 (0)	70 (23)
Donovan[101]	2020	Vietnam	High	Cohort	204	NA	NA	43 (21)	0 (0)	113 (55)
Jarvis[105]	2019	Botswana	Low	Cross-Sectional	138	38 (5-90)	80 (58)	97 (70)	3 (2.2)	7 (5.1)
van Laarhoven[33]	2017	Indonesia	High	Cohort	761	30 (14-78)	460 (60)	146 (19)	0 (0)	339 (45)
Dendane[18]	2013	Morocco	Low	Cohort	414	32 (14-84)	221 (53)	1 (0.2)	0 (0)	246 (60)
Metcalf[106]	2018	Peru	Low	Cohort	37	40 (19-77)	27 (73)	23 (62)	11 (30)	8 (22)
Jipa[107]	2017	Romania	Low	Cohort	111	34 (18-75)	57 (51)	32 (29)	0 (0)	20 (18)
Bateman[102]	2012	South Africa	High	Cohort	93	32 (15-71)	43 (46)	49 (53)	9 (9.7)	30 (32)
Boulware[103]	2014	South Africa, Uganda	High, Low	RCT	61	35 (19-75)	37 (61)	58 (95)	4 (7)	31 (51)
Rhein[104]	2019	Uganda	Low	RCT	586	34 (14-75)	343 (59)	581 (99)	24 (4)	98 (17)
TOTAL					**3671**	**35 (5-90)**	**1746 (58)**	**1644 (45)**	**104 (2.8)**	**1148 (31)**

*RCT = Randomized controlled trial

Figure 3.2. Meningitis Etiologies of Participants

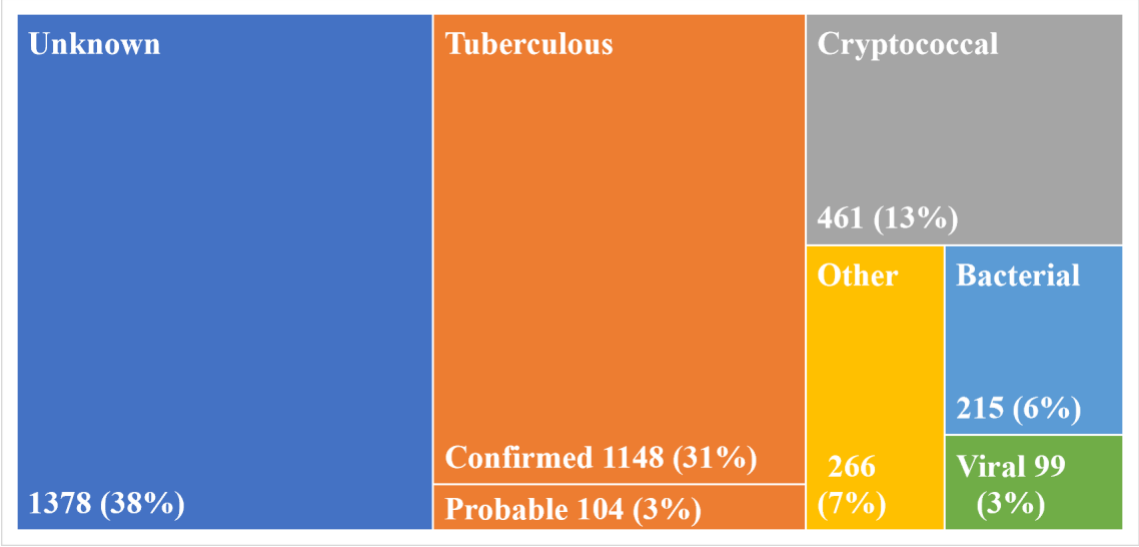


Table 3.3. Univariate analysis of clinical, hematological, and CSF data of individual participants with and without TB meningitis.

	non-TBM (N=2419)	TBM (N=1252)	p-value*
	Median (IQR) or N (%)	Median (IQR) or N (%)	
Age, years	35 (27-46)	32 (25-43)	<0.001
Men	1,229 (59)	517 (57)	0.260
Symptom Duration, days	7 (4-21)	11 (6-20)	<0.001
Fever	1,298 (54%)	871 (70%)	<0.001
HIV-positive	1,225 (51%)	419 (34%)	<0.001
Blood Glucose, mg/dL	103 (94-113)	104 (84-120)	0.477
CSF WBC count, cells/mm^3	8 (2.5-139)	140 (40-319)	<0.001
WBC Differential			<0.001
WBC < 5 cells/mm^3	1,105 (46)	125 (10)	
Neutrophilic Dominance	373 (15)	332 (27)	
Lymphocytic Dominance	941 (39)	795 (64)	
CSF Protein, mg/dL	60 (31-134)	154 (86-267)	<0.001
CSF Glucose, mg/dL	53 (37-68)	23 (5.5-41)	<0.001
CSF CrAg Positive	455 (19)	13 (1)	<0.001

*p-value based on chi-square or Kruskal-Wallis Rank Sum test

Multivariable Prediction Models

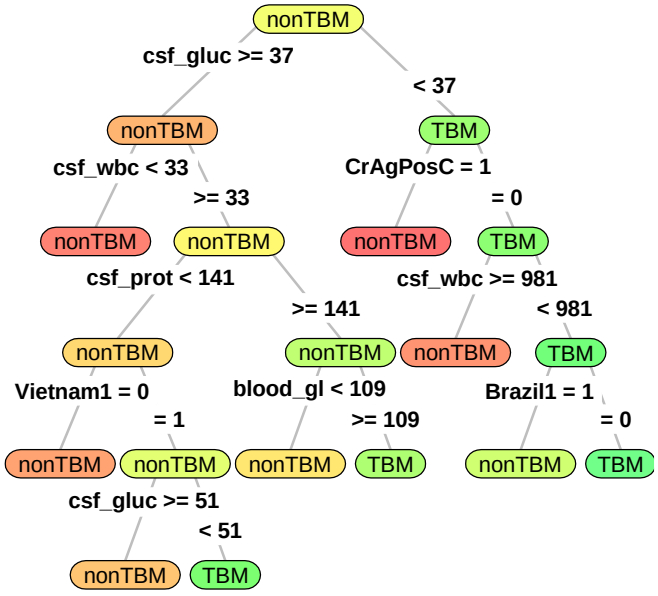
Backwards stepwise predictor selection revealed CSF WBC count, CSF WBC differential, CSF glucose, blood glucose, HIV status, and CSF cryptococcal antigen (CrAg) as significant predictors of TB meningitis (**Table 3.4**). Stratified intercepts for each country (with Brazil as the reference group) indicated significant heterogeneity in predictors from Indonesia, Morocco, Peru, South Africa, Uganda, and Vietnam, with an overall increased odds of TB meningitis in these countries (**Table 3.4**). A logistic regression model without stratified intercepts for country is presented in **Appendix E**.

Table 3.4. Logistic Regression Coefficients

Covariate	Odds Ratio (95% CI)
CSF WBC count, 100/mm^3	0.91 (0.90-0.93)
WBC < 5 cells/mm^3	0.25 (0.16-0.40)
Neutrophilic Dominance	0.96 (0.59-1.56)
Lymphocytic Dominance	1.00 (0.64-1.57)
CSF Glucose, 10 mg/dL	0.67 (0.64-0.71)
Blood Glucose, 10 mg/dL	1.07 (1.03-1.10)
HIV-positive	1.43 (1.13-1.82)
CSF CrAg Positive	0.02 (0.01-0.04)
Botswana	0.62 (0.32-1.29)
Indonesia	2.77 (2.02-3.80)
Morocco	5.04 (3.15-8.09)
Peru	2.68 (1.26-5.72)
Romania	0.89 (0.50-1.60)
South Africa	2.41 (1.43-4.07)
Uganda	4.93 (3.44-7.08)
Vietnam	5.52 (3.98-7.64)
Brazil	(ref)

With the exception of symptom duration, blood WBC count, age, and biological sex, all key predictors were used in the development of both CART and random forest models. Symptom duration, blood WBC count, age, and biological sex were excluded due to complete missingness within datasets, which could not be imputed. The resulting CART decision tree is shown in **Figure 3.3**. The color gradient corresponds to predictive

Figure 3.3. Classification and Regression Tree (CART)



probability of TB meningitis with green representing higher probability of TB meningitis and red representing lower probability of TB meningitis. The random forest model indicated that CSF glucose, CSF WBC count, CSF protein, and blood glucose were the most important variables used in model development (**Appendix F**).

Internal-External Cross-Validation

Model performance was assessed in each of the 12 datasets (folds) left out in the IECV process. All MPMs demonstrated poor calibration in each fold (**Table 3.5**). The CART MPM had the widest range of E/O and ROC values, indicating inconsistent performance across the study IPD. Additionally, CART had the lowest range of ROC values (0.60-0.84), indicating a weaker discriminatory power than logistic and random forest models. Performance was worst across all three models for Metcalf.[106] Performance was consistent across all three MPMs for Azevedo,[97] Gualberto,[96] Dendane,[18] and the Uganda dataset[103,104] indicating clearer distinction between TB and non-TB meningitis cases (lower heterogeneity). Performance was most inconsistent in

van Laarhoven,[33] Bateman,[102] Jipa,[107] the Vietnam dataset[99-101] indicating more heterogeneity in TB meningitis cases and non-cases.

Table 3.5. Internal-External Cross-Validation Multivariable Prediction Model Performance in Test Dataset

Test Dataset	Logistic			CART			Random Forest		
	E/O	Slope	ROC	E/O	Slope	ROC	E/O	Slope	ROC
van Laarhoven[33]	0.07	-0.45	0.77	1.15	0	0.60	0.98	0.49	0.89
Bateman[102]	1.87	0.73	0.79	1.03	0.23	0.73	0.90	0.35	0.83
Jipa[107]	0.05	-0.22	0.90	2.75	0.07	0.79	1.85	0.70	0.66
Azevedo[97]	0.17	-0.20	0.79	0.08	-0.30	0.84	0.42	0.09	0.86
de Almeida[98]	0.58	0.05	0.74	2.15	0.06	0.79	1.31	0.19	0.72
Anselmo[95]	0.04	-0.44	0.66	0	-0.39	0.80	0.02	-0.44	0.84
Gualberto[96]	0.50	-0.02	0.88	0.29	-0.07	0.77	0.71	-0.03	0.89
Metcalf[106]	0.21	-0.59	0.64	1.00	-0.33	0.61	0.84	-0.09	0.65
Dendane[18]	0.05	-0.23	0.85	1.28	0.06	0.83	1.21	0.35	0.82
Vietnam[99-101]	0.08	-0.33	0.84	0.73	0.17	0.68	0.64	0.54	0.85
Uganda[103,104]	0.24	0.06	0.85	0.60	0.04	0.79	0.49	0.29	0.83
Jarvis[105]	0.40	-0.09	0.78	2.30	0.09	0.83	2.00	0.05	0.85

Overall discriminatory power measured by the mean area under the ROC curve was similar across MPMs, with a ROC value of 0.79 (95% CI; 0.75-0.83) for logistic, 0.76 (95% CI; 0.71-0.80) for CART, and 0.81 (95% CI; 0.76-0.85) for random forest (**Figure 3.4**).

Figure 3.4. ROC Curves for Logistic, CART, and Random Forest MPMs

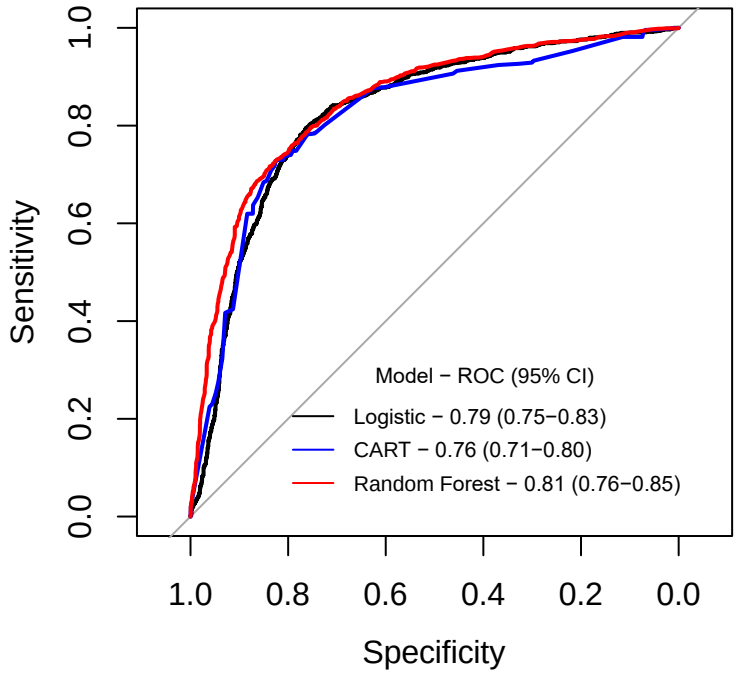


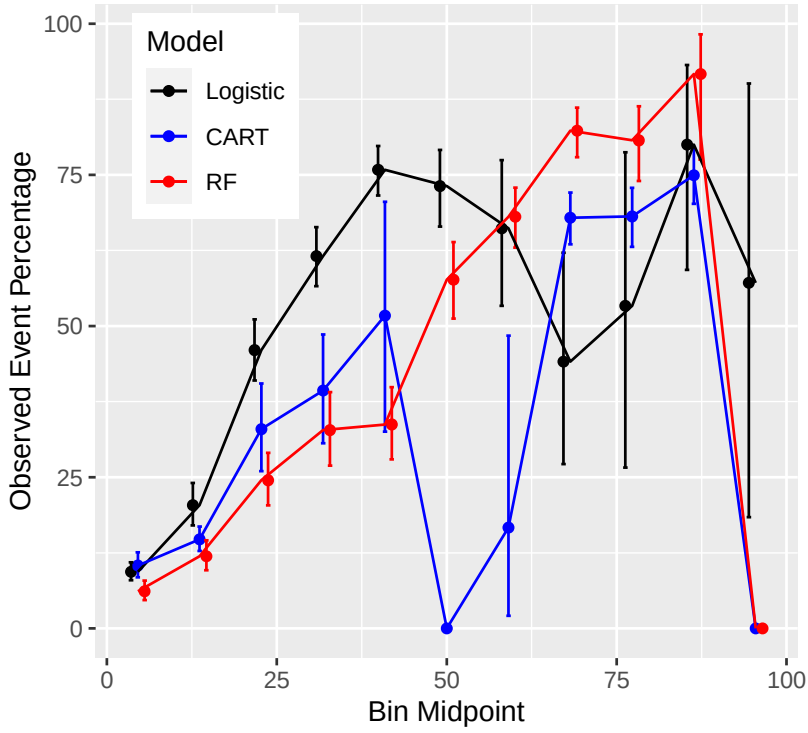
Table 3.6. Overall Performance of Logistic, CART, and Random Forest MPMs

MPM	Overall Calibration			Brier Score	ROC (95% CI)
	Ratio (E/O)[1]	Intercept	Slope		
Logistic	0.16	30.6	0.45	0.19	0.79 (0.75-0.83)
CART	0.99	18.4	0.32	0.16	0.76 (0.71-0.80)
Random Forest	0.86	14.9	0.59	0.15	0.81 (0.76-0.85)

[1]E/O = Expected (predicted) case classification / observed case classification

The Brier score was also similar across the three models with the logistic model performing only 0.03 points above the CART MPM. Conversely, calibration was varied between the MPMs. The logistic MPM had the worst calibration with the E/O ratio and slope values furthest from one compared to the CART and random forest MPMs (**Table 3.6**). Of the three models, calibration slope was closest to one for the random forest model (0.59). Visual inspection of the calibration plots for all three MPMs shows a clear hierarchy of calibration performance with random forest outperforming both logistic and CART MPMs (**Figure 3.5**). Random forest had the most bin midpoints falling along a 45-degree line (grey dotted line), demonstrating that the number of TB meningitis cases increases as prediction probability increases.

Figure 3.5. Calibration Plot for Logistic, CART, and Random Forest MPMs



CART was the most poorly calibrated model with the widest confidence intervals and no observed events falling in the 50-bin midpoint (**Figure 3.5**).

Diagnostic utility of each MPM is summarized in **Tables 3.7-3.9**. At the pre-determined prediction probability cutoff of 0.1, sensitivity and specificity were 0.84 (95% CI; 0.76-0.92) and 0.52 (95% CI; 0.41-0.64) for logistic regression, 0.77 (95% CI; 0.68-0.87) and 0.59 (95% CI;0.45-0.73) for CART, and 0.89 (95% CI; 0.82-0.97) and 0.36 (95% CI; 0.24-0.48) for random forest, respectively. The random forest MPM missed the fewest number of TB meningitis cases with a FNR of 0.11 (95%CI; 0.03-0.18), compared to the logistic, 0.16 (95%CI; 0.08-0.24), and CART, 0.23 (95%CI; 0.13-0.32), MPMs. In the presence of a negative test, the logistic and random forest MPMs decreased the probability of TB meningitis disease by approximately 30% (LR- = 0.25, 95%CI; 0.13-0.35 and LR- = 0.23, 95%CI; 0.11-0.35, respectively). The CART model had the highest proportion correctly classified with 0.69 (95%CI; 0.61-0.77) of predictions correctly classifying IPD as either TB or non-TB meningitis followed by logistic, 0.65 (95%CI; 0.59-0.72), and random forest, 0.56 (95%CI; 0.49-0.62).

Table 3.7. Logistic Regression Diagnostic Performance in IECV by Probability Cutoff

Cutoff for T+	Proportion T+	Sensitivity (T+|D+)	Specificity (T-|D-)	Positive Predictive Value (D+|T+)	Negative Predictive Value (D-|T-)	False Positive Rate (T+|D-)	False Negative Rate (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	Proportion Correctly Classified
0	1 (1-1)	1 (1-1)	0 (0-0)	0.29 (0.19-0.39)	NA	1 (1-1)	0 (0-0)	1 (1-1)	NA	0.29 (0.19-0.39)
0.1	0.58 (0.45-0.71)	0.84 (0.76-0.92)	0.52 (0.41-0.64)	0.41 (0.31-0.52)	0.93 (0.90-0.96)	0.48 (0.36-0.59)	0.16 (0.08-0.24)	2.2 (1.6-2.7)	0.25 (0.15-0.35)	0.65 (0.59-0.72)
0.2	0.43 (0.29-0.56)	0.74 (0.64-0.85)	0.70 (0.58-0.82)	0.51 (0.40-0.62)	0.90 (0.86-0.94)	0.30 (0.18-0.42)	0.26 (0.15-0.36)	3.6 (2.4-4.9)	0.33 (0.22-0.44)	0.76 (0.71-0.81)
0.3	0.32 (0.18-0.46)	0.62 (0.50-0.74)	0.79 (0.68-0.91)	0.60 (0.49-0.71)	0.85 (0.78-0.93)	0.21 (0.09-0.32)	0.38 (0.26-0.50)	7 (3-11)	0.45 (0.32-0.57)	0.78 (0.72-0.84)
0.4	0.22 (0.1-0.34)	0.41 (0.28-0.53)	0.86 (0.76-0.97)	0.62 (0.52-0.73)	0.80 (0.72-0.88)	0.14 (0.03-0.24)	0.59 (0.47-0.72)	7.8 (4.3-11)	0.65 (0.52-0.78)	0.77 (0.7-0.84)
0.5	0.10 (0-0.22)	0.19 (0-0.33)	0.93 (0.83-1)	0.69 (0.57-0.81)	0.75 (0.65-0.85)	0.07 (0-0.17)	0.81 (0.67-0.94)	11 (1.8-20)	0.83 (0.71-0.95)	0.72 (0.63-0.82)
0.6	0.07 (0-0.17)	0.11 (0-0.24)	0.94 (0.87-1)	0.42 (0.24-0.61)	0.74 (0.64-0.84)	0.06 (0-0.13)	0.89 (0.76-1)	6.1 (1.1-11)	0.91 (0.78-1)	0.71 (0.62-0.81)
0.7	0.05 (0-0.12)	0.07 (0-0.19)	0.97 (0.92-1)	0.41 (0.15-0.67)	0.73 (0.63-0.83)	0.03 (0-0.08)	0.93 (0.81-1.1)	0.79 (0.07-1.5)	0.94 (0.84-1)	0.72 (0.62-0.82)
0.8	0.03 (0-0.09)	0.05 (0-0.14)	0.98 (0.96-1)	0.71 (0.62-0.80)	0.72 (0.63-0.82)	0.02 (0-0.04)	0.95 (0.86-1)	3.3 (2.7-5.2)	0.96 (0.88-1)	0.72 (0.63-0.82)
0.9	0.01 (0-0.03)	0.02 (0-0.048)	0.99 (0.98-1)	0.67 (0.61-0.70)	0.71 (0.61-0.81)	0.01 (0-0.02)	0.98 (0.95-1)	2.8 (1.3-4.2)	0.99 (0.97-1)	0.71 (0.62-0.81)
1	0 (0-0)	0 (0-0)	1 (1-1)	NA	0.71 (0.61-0.81)	0 (0-0)	1 (1-1)	NA	1 (1-1)	0.71 (0.61-0.81)

Table 3.8. CART Diagnostic Performance in IECV by Probability Cutoff

Cutoff for T+	Proportion T+	Sensitivity (T+|D+)	Specificity (T-|D-)	Positive Predictive Value (D+|T+)	Negative Predictive Value (D-|T-)	False Positive Rate (T+|D-)	False Negative Rate (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	Proportion Correctly Classified
0	1 (1-1)	1 (1-1)	0 (0-0)	0.29 (0.19-0.39)	NA	1 (1-1)	0 (0-0)	1 (1-1)	NA	0.29 (0.19-0.39)
0.1	0.51 (0.37-0.64)	0.77 (0.68-0.87)	0.59 (0.45-0.73)	0.44 (0.34-0.54)	0.87 (0.8-0.94)	0.41 (0.27-0.55)	0.23 (0.13-0.32)	2.5 (1.8-3.2)	0.39 (0.27-0.51)	0.69 (0.61-0.77)
0.2	0.43 (0.31-0.54)	0.72 (0.63-0.82)	0.70 (0.6-0.8)	0.48 (0.37-0.60)	0.88 (0.81-0.94)	0.30 (0.20-0.40)	0.28 (0.18-0.37)	3 (2.3-3.8)	0.38 (0.27-0.49)	0.73 (0.66-0.80)
0.3	0.35 (0.23-0.46)	0.66 (0.57-0.76)	0.79 (0.71-0.86)	0.55 (0.44-0.66)	0.87 (0.81-0.93)	0.21 (0.14-0.29)	0.34 (0.24-0.43)	4.3 (3-5.7)	0.41 (0.31-0.51)	0.78 (0.74-0.83)
0.4	0.30 (0.17-0.43)	0.52 (0.34-0.69)	0.81 (0.72-0.9)	0.49 (0.34-0.64)	0.84 (0.78-0.91)	0.19 (0.10-0.28)	0.48 (0.31-0.66)	3.4 (1.9-4.9)	0.56 (0.39-0.73)	0.78 (0.73-0.83)
0.5	0.30 (0.17-0.43)	0.52 (0.34-0.69)	0.81 (0.72-0.9)	0.49 (0.34-0.64)	0.84 (0.78-0.91)	0.19 (0.10-0.28)	0.48 (0.31-0.66)	3.4 (1.9-4.9)	0.56 (0.39-0.73)	0.78 (0.73-0.83)
0.6	0.30 (0.17-0.43)	0.51 (0.33-0.69)	0.81 (0.73-0.90)	0.54 (0.42-0.67)	0.84 (0.78-0.91)	0.19 (0.10-0.27)	0.49 (0.31-0.67)	3.8 (2.3-5.4)	0.56 (0.39-0.74)	0.78 (0.73-0.83)
0.7	0.25 (0.13-0.38)	0.48 (0.30-0.66)	0.86 (0.78-0.93)	0.62 (0.51-0.74)	0.84 (0.77-0.91)	0.14 (0.07-0.22)	0.52 (0.34-0.7)	5.2 (3.5-6.9)	0.57 (0.40-0.75)	0.80 (0.75-0.85)
0.8	0.04 (0-0.11)	0.07 (0-0.18)	0.98 (0.95-1)	0.83 (0.76-0.91)	0.73 (0.64-0.83)	0.02 (0-0.05)	0.93 (0.82-1)	8.2 (4.7-12)	0.94 (0.83-1)	0.73 (0.64-0.83)
0.9	0 (0-0)	0 (0-0)	1 (1-1)	NA	0.71 (0.61-0.81)	0 (0-0)	1 (1-1)	NA	1 (1-1)	0.71 (0.61-0.81)
1	0 (0-0)	0 (0-0)	1 (1-1)	NA	0.71 (0.61-0.81)	0 (0-0)	1 (1-1)	NA	1 (1-1)	0.71 (0.61-0.81)

Table 3.9. Random Forest Diagnostic Performance in IECV by Probability Cutoff

Cutoff for T+	Proportion T+	Sensitivity (T+|D+)	Specificity (T-|D-)	Positive Predictive Value (D+|T+)	Negative Predictive Value (D-|T-)	False Positive Rate (T+|D-)	False Negative Rate (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	Proportion Correctly Classified
0	1 (1-1)	1 (1-1)	0 (0-0)	0.29 (0.19-0.39)	NA	1 (1-1)	0 (0-0)	1 (1-1)	NA	0.29 (0.19-0.39)
0.1	0.70 (0.57-0.82)	0.89 (0.82-0.97)	0.36 (0.24-0.48)	0.35 (0.26-0.45)	0.93 (0.88-0.98)	0.64 (0.52-0.76)	0.11 (0.03-0.18)	1.5 (1.3-1.8)	0.23 (0.11-0.35)	0.56 (0.49-0.62)
0.2	0.51 (0.36-0.66)	0.80 (0.70-0.91)	0.58 (0.43-0.73)	0.46 (0.37-0.55)	0.90 (0.84-0.97)	0.42 (0.27-0.57)	0.20 (0.09-0.30)	3 (1.9-4.1)	0.30 (0.16-0.44)	0.70 (0.64-0.77)
0.3	0.41 (0.27-0.56)	0.72 (0.61-0.83)	0.69 (0.54-0.84)	0.54 (0.43-0.65)	0.84 (0.76-0.92)	0.31 (0.16-0.46)	0.28 (0.17-0.39)	6.2 (1.4-11)	0.42 (0.31-0.52)	0.75 (0.69-0.81)
0.4	0.34 (0.20-0.47)	0.62 (0.49-0.75)	0.78 (0.67-0.89)	0.60 (0.48-0.73)	0.87 (0.80-0.93)	0.22 (0.11-0.33)	0.38 (0.25-0.51)	4.9 (2.9-6.9)	0.44 (0.31-0.58)	0.78 (0.73-0.83)
0.5	0.27 (0.15-0.38)	0.52 (0.39-0.66)	0.86 (0.79-0.92)	0.65 (0.52-0.77)	0.84 (0.78-0.90)	0.14 (0.08-0.21)	0.48 (0.34-0.61)	5.9 (3.9-8)	0.53 (0.40-0.66)	0.80 (0.77-0.84)
0.6	0.16 (0.09-0.24)	0.35 (0.23-0.47)	0.93 (0.89-0.96)	0.66 (0.55-0.77)	0.78 (0.71-0.86)	0.07 (0.04-0.11)	0.65 (0.53-0.77)	8.8 (4.4-13)	0.69 (0.58-0.80)	0.79 (0.73-0.84)
0.7	0.06 (0-0.10)	0.17 (0-0.26)	0.98 (0.97-0.99)	0.64 (0.45-0.82)	0.74 (0.64-0.83)	0.02 (0.01-0.03)	0.83 (0.74-0.93)	6.4 (3.7-9.2)	0.85 (0.76-0.94)	0.75 (0.66-0.83)
0.8	0 (0-0.02)	0.02 (0-0.05)	1 (1-1)	0.79 (0.58-1)	0.72 (0.62-0.81)	0 (0-0.01)	0.98 (0.95-1)	8.9 (2.7-15)	0.98 (0.95-1)	0.72 (0.62-0.82)
0.9	0 (0-0)	0 (0-0)	1 (1-1)	NA	0.71 (0.61-0.81)	0 (0-0)	1 (1-1)	NA	1 (1-1)	0.71 (0.61-0.81)
1	0 (0-0)	0 (0-0)	1 (1-1)	NA	0.71 (0.61-0.81)	0 (0-0)	1 (1-1)	NA	1 (1-1)	0.71 (0.61-0.81)

Discussion

In this systematic review and IPD meta-analysis we developed and internally validated three unique MPMs using IPD from over three thousand adults spanning nine countries. Our data clearly demonstrates that heterogeneity in clinical and subacute TB meningitis case presentation impacts performance of MPMs for TB meningitis. Although we accounted for this heterogeneity in every step of MPM development – from the data we used to the analytical methods employed – IECV revealed that performance of the three MPMs varied considerably in different populations and settings. Random forest, a machine learning based MPM, had slightly more promise in reducing the number of missed TB meningitis cases with a sensitivity of 0.89 (95% CI; 0.82-0.97) and a false negative rate of 0.11 (95%CI; 0.03-0.18) at a threshold of 0.1. Our MPMs and internal validation results are in concordance with the 10 published clinical prediction tools based on MPMs, which showed similar significant predictors of TB meningitis and performance, respectively. Our study expands on the current literature through the use of IPD from multiple populations and settings and machine learning modeling approaches to improve the timeliness and efficiency of TB meningitis diagnosis.

We have demonstrated that machine learning approaches to MPM development are not superior to classic MPM development approaches such as logistic regression. In fact, the CART MPM performed substantially worse than the logistic MPM and the random forest MPM performance was no different than the logistic regression MPM. Machine learning algorithms have been gaining popularity as an alternative approach to prediction and classification algorithms in clinical contexts. However, a recent systematic review indicates that there is, on average, no difference in the performance between

logistic regression and machine learning approaches.[108] The strong predictive ability of machine learning models increase the susceptibility of over-calibration to the development dataset and thus impact performance external validity.[108] This phenomenon was demonstrated in the CART MPM where over-calibration was observed in seven out of the twelve folds and had the worst overall performance compared to the logistic and random forest MPMs. Additionally, machine learning approaches tend to perform better when the amount of relevant information is greater than the amount of non-relevant information, otherwise known as the signal-to-noise ratio. In in the case of TB meningitis, there is a considerable amount of observed "noise" due to case-mix variation, lowering the overall signal-to-noise ratio. The non-inferiority in the performance of the logistic MPM compared to the random forest MPM demonstrates that the two machine learning approaches evaluated in this study do not improve prediction for TB meningitis, which is already challenging to diagnose.

Secondly, our data demonstrates the significant contribution HIV-infection makes to case-mix variation. HIV prevalence has been identified as a significant contributor to heterogenous outcomes in TB meningitis and is thought to modulate pathogenesis of TB meningitis disease.[5] All three MPMs developed in this study performed most consistently in IPD studies that had the entire sample either comprised of HIV-positive[96,97,103,104] or -negative persons.[18] Conversely, the MPMs were most inconsistent in IPD studies with an HIV-prevalence range from 13-53%. Although HIV status was included in the MPMs as an independent predictor of TB meningitis, there remains significant heterogeneity in performance in IPD datasets with a mix of HIV-positive and -negative persons. In a separate analysis we excluded the country intercepts and found that HIV-status was no

longer an independent predictor of TB meningitis. This finding suggests that the country intercepts in the logistic regression model attempt to explain the heterogeneity induced by the presence of HIV-infection. Further research is needed to elucidate the relationship between HIV prevalence, geographic location, and TB meningitis prediction.

While this analysis has many strengths that make it an important contribution to TB meningitis diagnostics, there are some limitations. It is possible that TB meningitis cases were misclassified as non-cases. This limitation stems from the fact that there is no "gold-standard" for diagnosing TB meningitis and the current diagnostic procedures have variable sensitivity and specificity, which is the primary motivation behind this study. We attempted to account for this limitation by standardizing our TB case definitions across the IPD studies and including cases of probable TB meningitis in the outcome. These steps likely captured the majority of TB cases.

Another limitation to our analysis is missing data, including a lack of outcome data on all participants. Due to complete missingness of symptom duration, age, and biological sex in some IPD studies, these variables could not be imputed and had to be excluded from MPM development. Each of these variables have been indicated in prior MPMs as significant predictors of TB meningitis but could not be explored in this analysis. The lack of outcome data prevents us from conducting an efficacy validation on survival, which would have illustrated any reduction in mortality as a result of early identification of TB meningitis. Although not a limiting factor in terms of MPM development, it does limit our ability to show how the implementation of the MPMs could be useful in preventing adverse outcomes.

In conclusion, a machine learning based MPM does not substantially improve TB diagnostics over logistic regression. TB meningitis remains a difficult disease to diagnose with HIV status contributing to underlying heterogeneity and case-mix variation. Our study was limited by the available data and extensive missingness. Prioritizing the collection of data important to predicting TB meningitis such as CSF WBC count, differential, and CSF glucose could be used to better inform a MPM and are rapid, cost-effective ways better predict TB meningitis.

CHAPTER 4. MANUSCRIPT 3: EXTERNAL VALIDATION OF CLINICAL PREDICTION TOOL FOR TUBERCULOUS MENINGITIS

Introduction

As discussed in prior sections, a major limitation to the clinical prediction tools based on multivariable prediction models (MPMs) that have been developed for TB meningitis diagnosis is that performance is variable in different populations[81] (See **Table 1.2** for full list of prior clinical prediction tools). A clinical prediction tool developed with data from a Vietnamese population is currently the only one to be tested for external validity.[12] This clinical prediction tool was originally described to be 86% sensitive and 79% specific for TB meningitis diagnosis in Vietnamese adults,[12] and subsequent studies in Turkey,[21] Vietnam,[111] India,[17] China,[22] and Colombia[23] reported sensitivities >90% and specificities ranging from 50–90%.[81] However, the performance of the Vietnamese clinical prediction tool was not generalizable to an HIV-positive cohort in Malawi, where the tool was only 78% sensitive and 43% specific, with cryptococcal meningitis accounting for most of the false-positive results.[24] The Uniform TB Meningitis Case Definition (UTBMCD) has been published to help standardize diagnostics in TB meningitis clinical research and enable direct comparison of studies.[10] However, the UTBMCD was not designed to be used as a clinical prediction tool and was shown to perform poorly when used in non-research settings.[112]

In the past, clinical prediction tools have been developed with relatively small datasets, which likely does not capture the complexities of predictor selection, limits the types of model approaches that are feasible, and/or leads to the model overfitting the data.[113] This not only has implications for internal validity but also impacts the ability for a model to be externally valid. To overcome this challenge, we have addressed sources of

bias in every step of model development from inclusion of data from different populations/settings to internally-externally cross-validating (IECV) the model with each external dataset we include (Manuscript 2). Externally validating the clinical prediction tool can further evaluate its diagnostic performance and demonstrate its clinical utility in various settings and populations.

The aim of this study is to assess performance of the MPMs developed in Manuscript 2 with data from an external cohort not used in the development of the MPMs. Additionally, we evaluated performance along different prediction probability thresholds to accomplish two different goals. First, we determined the prediction probability threshold that maximizes sensitivity in order to more efficiently rule out TB meningitis. This is important because most confirmatory diagnostic tests for TB meningitis lack sensitivity to rule out TB meningitis, which can miss cases of TB meningitis and further delay treatment. Secondly, we identified the probability thresholds for which we could categorize clinically actionable next steps, such as the threshold for which TB meningitis can be ruled out, when clinicians should seek confirmatory testing (e.g., Gene Xpert, TB culture, etc.), and the threshold for which clinicians can immediately begin anti-TB meningitis treatment. This will also improve efficiency and help guide clinical decision making.

Methods

We attained screening data from a multisite, observational cohort based in Uganda entitled, *Improving Diagnostics and Neurocognitive Outcomes in HIV/AIDS-related Meningitis* (DINEOUT). Participants were included if they had complete data for the predictors included in the clinical prediction tool developed in Manuscript 2: cerebral

spinal fluid (CSF) white blood cell (WBC) count, CSF WBC differential, CSF glucose, CSF protein, blood glucose, HIV status, and CSF cryptococcal antigen (CrAg) test results. We performed single imputation of the median value for missing blood glucose.

We defined 'confirmed' TB meningitis as having any of the following positive tests in the CSF: Xpert MTB/RIF (Cepheid, Sunnyvale, CA), Xpert Ultra MTB/RIF, other PCR, culture, acid fast bacilli (AFB), or 'definite' TB meningitis classification per the Uniform TBM case definition.[10] We defined 'probable' TB meningitis as having no alternate diagnoses and any CT, MRI, or X-ray suggestive of TB meningitis or a 'probable' TB meningitis case classification per the UTBMCD.[10]

Data Analysis

Baseline clinical characteristics and demographic data was compared by TB meningitis case status using chi-squared or non-parametric methods as indicated. We assessed diagnostic performance using calibration ratio of predicted (expected) to observed outcomes (denoted by E/O), calibration plots (intercept and slope), the area under the receiver operating characteristic (ROC) curve, and the Brier Score.[87,93,94] We calculated sensitivity (SE), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), false positive rate (FPR), false negative rate (FNR), positive likelihood ratio (LR+), negative likelihood ratio (LR-), and proportion correctly classified (PCC) along different prediction thresholds. All analyses were conducted in R studio.

Results

Participants Included

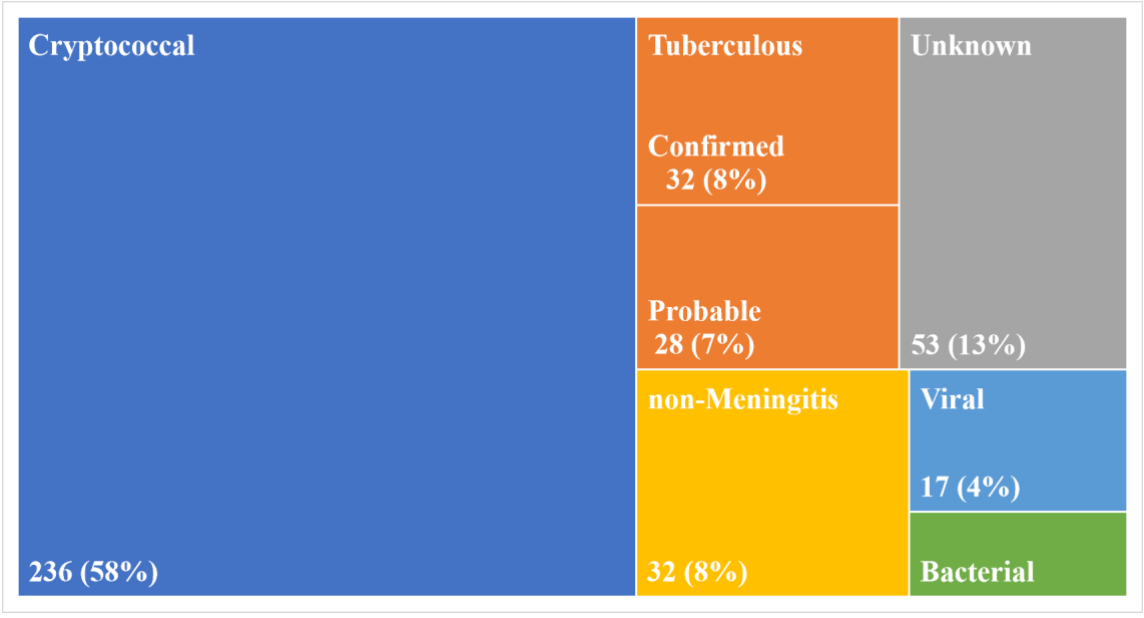
A total of 404 participants were included in the external validation dataset from DINEOUT (**Table 4.1**). Of those, 32 (8%) participants met the case definition for

Table 4.1. Univariate Analysis, clinical, hematological, and CSF data of individual participants in external validation dataset

	non-TBM (N=344)	TBM (N=60)	p-value*
	Median (IQR) or N (%)	Median (IQR) or N (%)	
Age, years	36 (30-43)	33 (27-39)	0.015
Men	214 (62)	33 (55)	0.361
Fever	155 (45)	55 (92)	<0.001
HIV-positive	332 (97)	54 (90)	0.055
Blood Glucose, mg/dL	120 (120-120)	120 (120-120)	<0.001
CSF WBC count, cells/mm^3	2.5 (2.5-31)	33 (2.5-178)	<0.001
WBC Differential			<0.001
WBC < 5 cells/mm^3	228 (66)	23 (38)	
Neutrophilic Dominance	4 (1)	3 (5)	
Lymphocytic Dominance	112 (33)	34 (57)	
CSF Protein, mg/dL	84 (40-119)	11 (60-169)	0.008
CSF Glucose, mg/dL	70 (47-90)	40 (20-76)	<0.001
CSF CrAg Positive	235 (68)	0 (0)	<0.001

*p-value based on chi-square or Kruskal-Wallis Rank Sum test

Figure 4.1. Meningitis Etiologies in External Validation Dataset



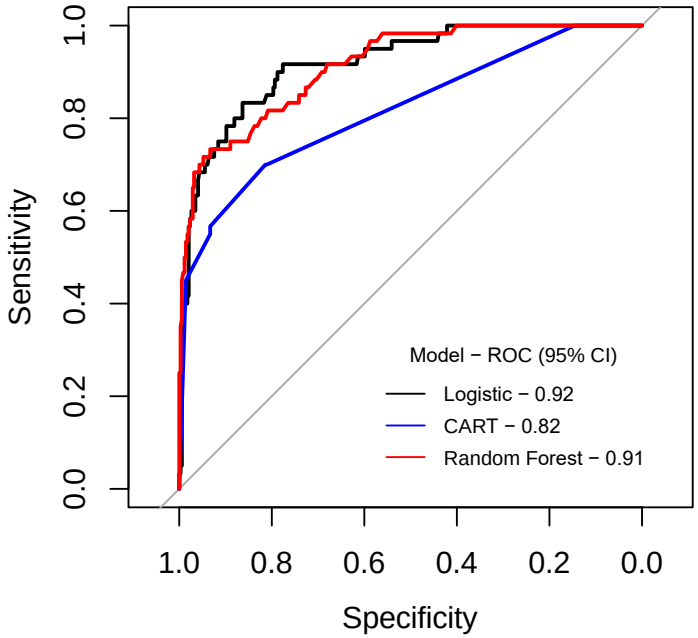
confirmed TB meningitis and 28 (7%) met the case definition for probable TB meningitis (**Figure 4.1**). The dominant meningitis etiology was cryptococcal meningitis (58%). The age range of participants was between 18 to 80 years old and was statistically

significantly different between TB and non-TB meningitis groups (**Table 4.1**). There was no difference in biological sex or gender between groups. Most participants were HIV-positive (N=386, 96%) and there was no significant difference in the proportion of HIV-positive individuals between the non-TB and TB meningitis groups (p<0.055).

Model Performance

The discriminatory power measured by the mean area under the ROC curve was highest for logistic regression (0.92), followed by random forest (0.90) and CART (0.85) (**Figure 4.2**). All models were under-calibrated with E/O ratio values less than one (**Table 4.2**). Of the three models, calibration slope was closest to one for the logistic regression model (0.94). Visual inspection of the calibration plots for all three MPMs shows that logistic regression has the most bin midpoints falling along a 45-degree line (grey dotted line) of observed event percentage indicating that logistic regression is the best calibrated MPM (**Figure 4.3**). CART

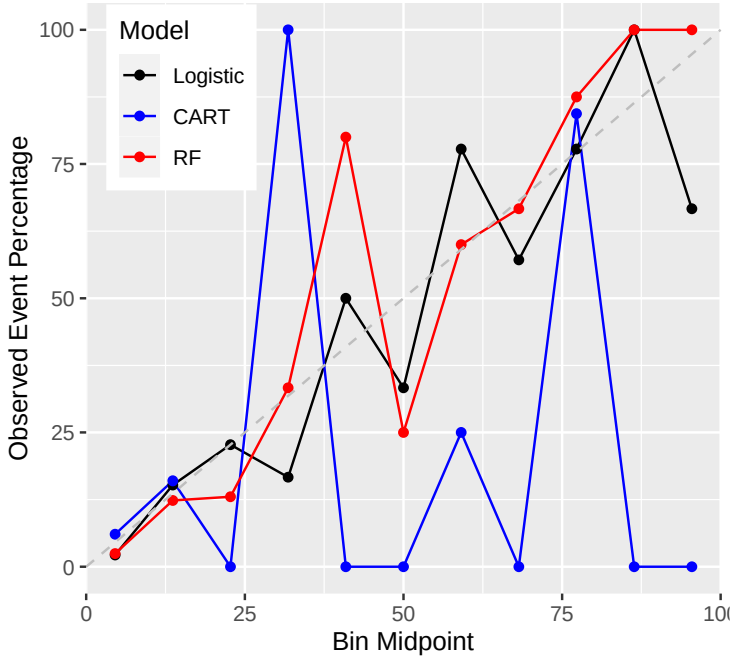
Figure 4.2. ROC Curves for Logistic, CART, and Random Forest MPMs



MPM	Overall Calibration			Brier Score	ROC
	Ratio (E/O)[1]	Intercept	Slope		
Logistic	0.73	0.46	0.94	0.07	0.92
CART	0.93	21.8	-0.02	0.09	0.82
Random Forest	0.70	-2.66	1.11	0.06	0.91

[1]E/O = Expected (predicted) case classification / observed case classification

Figure 4.3. Calibration Plot for Logistic, CART, and Random Forest MPMs



remained the least calibrated model. Brier scores were very similar across the three MPMs (**Table 4.2**). Diagnostic utility of each MPM is summarized in **Tables 4.3-4.5**. At the pre-determined prediction probability cutoff of 0.1, sensitivity and specificity were 0.90 and 0.79 for logistic regression, 0.70 and 0.81 for CART, and 0.87 and 0.73 for random forest, respectively. The logistic MPM missed the fewest number of TB meningitis cases with a FNR of 0.10 compared to the CART (FNR=0.30) and random forest (FNR=0.13) MPMs. In the presence of a negative test, the logistic MPM decreased the probability of TB meningitis disease by approximately 87% (LR- = 0.13) compared to ~40% and ~80% in the CART and random forest MPMs, respectively. The logistic model had the highest proportion correctly classified with 80% of predictions correctly classifying IPD as either TB or non-TB meningitis followed by CART, 79%, and random forest, 75%.

Table 4.3. Logistic Regression Diagnostic Performance in External Validation by Probability Cutoff

Cutoff for T+	% T+	SE (T+|D+)	SP (T-|D-)	PPV (D+|T+)	NPV (D-|T-)	FPR (T+/D-)	FNR (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	PCC
0	1	1	0	0.15	NA	1	0	1	NA	0.15
0.1	0.31	0.90	0.79	0.43	0.98	0.21	0.10	4.24	0.13	0.80
0.2	0.19	0.75	0.90	0.58	0.95	0.10	0.25	7.82	0.28	0.88
0.3	0.16	0.70	0.94	0.67	0.95	0.06	0.30	11.47	0.32	0.90
0.4	0.14	0.68	0.96	0.73	0.95	0.04	0.32	15.67	0.33	0.92
0.5	0.11	0.58	0.97	0.80	0.93	0.03	0.42	22.3	0.43	0.92
0.6	0.10	0.53	0.98	0.82	0.92	0.02	0.47	26.21	0.48	0.91
0.7	0.08	0.42	0.98	0.81	0.91	0.02	0.58	23.89	0.59	0.90
0.8	0.05	0.28	0.99	0.89	0.89	0.01	0.72	48.73	0.72	0.89
0.9	0.02	0.13	0.99	0.80	0.87	0.01	0.87	22.93	0.87	0.87
1	0	0	1	NA	0.85	0	1	NA	1	0.85

SE, Sensitivity; SP, Specificity; PPV, positive predictive value; NPV, negative predictive value; FPR, false positive rate; FNR, false negative rate; LR+, positive likelihood ratio; LR- negative likelihood ratio; PCC, proportion correctly classified

Table 4.4. CART Diagnostic Performance in External Validation by Probability Cutoff

Cutoff for T+	% T+	SE (T+|D+)	SP (T-|D-)	PPV (D+|T+)	NPV (D-|T-)	FPR (T+|D-)	FNR (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	PCC
0	1	1	0	0.15	NA	1	0	1	NA	0.15
0.1	0.26	0.70	0.81	0.39	0.94	0.19	0.30	3.7	0.37	0.79
0.2	0.14	0.57	0.93	0.60	0.93	0.07	0.43	8.48	0.46	0.88
0.3	0.14	0.57	0.93	0.60	0.93	0.07	0.43	8.48	0.46	0.88
0.4	0.14	0.55	0.93	0.59	0.92	0.07	0.45	8.23	0.48	0.88
0.5	0.14	0.55	0.93	0.59	0.92	0.07	0.45	8.23	0.48	0.88
0.6	0.08	0.45	0.99	0.84	0.91	0.01	0.55	30.96	0.56	0.91
0.7	0.08	0.45	0.99	0.84	0.91	0.01	0.55	30.96	0.56	0.91
0.8	0	0	1	NA	0.85	0	1	NA	1	0.85
0.9	0	0	1	NA	0.85	0	1	NA	1	0.85
1	0	0	1	NA	0.85	0	1	NA	1	0.85

SE, Sensitivity; SP, Specificity; PPV, positive predictive value; NPV, negative predictive value; FPR, false positive rate; FNR, false negative rate; LR+, positive likelihood ratio; LR- negative likelihood ratio; PCC, proportion correctly classified

Table 4.5. Random Forest Diagnostic Performance in External Validation by Probability Cutoff

Cutoff for T+	% T+	SE (T+|D+)	SP (T-|D-)	PPV (D+|T+)	NPV (D-|T-)	FPR (T+|D-)	FNR (T-|D+)	LR+ (T+|D+)/ (T+|D-)	LR- (T-|D+)/ (T-|D-)	PCC
0	1	1	0	0.15	NA	1	0	1	NA	0.15
0.1	0.36	0.87	0.73	0.36	0.97	0.27	0.13	3.17	0.18	0.75
0.2	0.17	0.73	0.93	0.64	0.95	0.07	0.27	10.09	0.29	0.90
0.3	0.14	0.68	0.96	0.75	0.95	0.04	0.32	16.79	0.33	0.92
0.4	0.12	0.65	0.97	0.80	0.94	0.03	0.35	22.36	0.36	0.92
0.5	0.10	0.57	0.98	0.81	0.93	0.02	0.43	24.37	0.44	0.92
0.6	0.09	0.53	0.98	0.84	0.92	0.02	0.47	30.58	0.47	0.92
0.7	0.07	0.43	0.99	0.93	0.91	0.01	0.57	74.53	0.57	0.91
0.8	0.04	0.25	1	1	0.88	0	0.75	NA	0.75	0.89
0.9	0.01	0.07	1	1	0.86	0	0.93	NA	0.93	0.86
1	0	0	1	NA	0.85	0	1	NA	1	0.85

SE, Sensitivity; SP, Specificity; PPV, positive predictive value; NPV, negative predictive value; FPR, false positive rate; FNR, false negative rate; LR+, positive likelihood ratio; LR- negative likelihood ratio; PCC, proportion correctly classified

Discussion

In this external validation study, we assessed the performance of three unique MPMs using IPD derived from a high HIV-prevalent setting. Our analysis clearly demonstrates predictive superiority of the logistic regression MPM compared to the CART and random forest machine learning approaches. The logistic MPM had better classification ability (ROC=0.92) and was better calibrated (slope=0.94) to the external validation dataset from DINEOUT. Although, the random forest MPM initially showed slightly more promise in reducing the number of missed TB meningitis cases in internal validation, poor calibration ultimately impacted external validity. Our MPMs performed as well as the 10 published clinical prediction tools based on MPMs, if not better.

This analysis identified important prediction thresholds that could be used to guide clinical practice for diagnosing TB meningitis. Across all three MPMs, the

predetermined prediction probability threshold of 0.10 was the best cutoff for excluding non-cases while maintaining a low false negative rate across all three MPMs. The logistic MPM performed best at this threshold with an NPV=0.98 and LR- = 0.13, indicating that a negative test (predicted probability <0.1) had substantially reduced the likelihood of a false negative test. The optimal threshold for 'ruling in' TB meningitis appeared to be ≥0.4, where the FPR = 0.04 and LR+ = 15.67, indicating that persons who had a positive test (predicted probability ≥0.4) substantially increased the likelihood of actually having TB meningitis. In practice, these predictive thresholds could be used as a guide to inform diagnostic approaches to meningitis. Anyone with a predictive probability less than 0.1 could be evaluated for another etiology and anyone with a predictive probability ≥0.4 could be started on anti-TB treatment immediately. Persons with a predictive probability between 0.1 and 0.4 could be referred for rapid confirmatory testing, such as an NAAT. In this diagnostic approach, there is a strong potential to reduce the delay between identification and treatment by increasing efficiency and maximizing resources.

A major strength of this analysis is the generalizability of our MPMs. Our MPMs showed good performance utilizing lab and clinical evaluations that are readily available in resource-limited settings, where the burden of TB meningitis is greatest. According to the WHO, blood glucose, HIV testing, and CrAg screenings are all considered essential diagnostics and are typically supplied in most hospitals and clinics. A full CSF evaluation may be harder to procure, but where there is capacity to attain a complete blood count and blood glucose values, there is capacity to attain these for CSF samples. In the absence of confirmatory diagnostic tests for TB meningitis, such as Gene Xpert or Gene

Xpert Ultra, our MPMs provide a tool to guide clinical decision making that can be utilized in most settings.

While this study has many strengths that make it an important contribution to improving TB meningitis diagnostics, there are some limitations. The limitation of misclassification of TB meningitis persists due to inadequate diagnostics. TB meningitis diagnosis is determined on a case-by-case basis by the clinical staff, including physicians, and principal investigators, using all the available data. Thus, this limitation is mitigated by experience and expertise of the clinical team who manages and treats the participants in the DINEOUT cohort. We are confident in the case classification determined by the DINEOUT team and anticipate that the proportion of misclassified cases would be negligible and not significantly impact the overall findings of this study. Another limitation to this study is representation from only one setting. Having data representing different locations and settings to externally validate the MPMs would have provided a more robust ascertainment of performance across different locations and settings. Finally, the DINEOUT cohort was compromised of a majority of HIV-positive persons. We observed in prior IECV that the MPMs performed best in cohort that were either comprised of mostly HIV positive or negative persons. Thus, the performance demonstrated in this analysis may be biased.

In conclusion, logistic regression prevailed over machine learning methods in accurately predicting TB meningitis. Developing MPMs that account for case-mix variation due to HIV-status and geographic location have improved the overall performance of clinical predictions tools for TB meningitis. Our study was limited by the available data for external validation. Incorporating the logistic MPM into clinical

evaluation for TB meningitis has the potential to be a cost-effective and efficient way to improve TB diagnostics.

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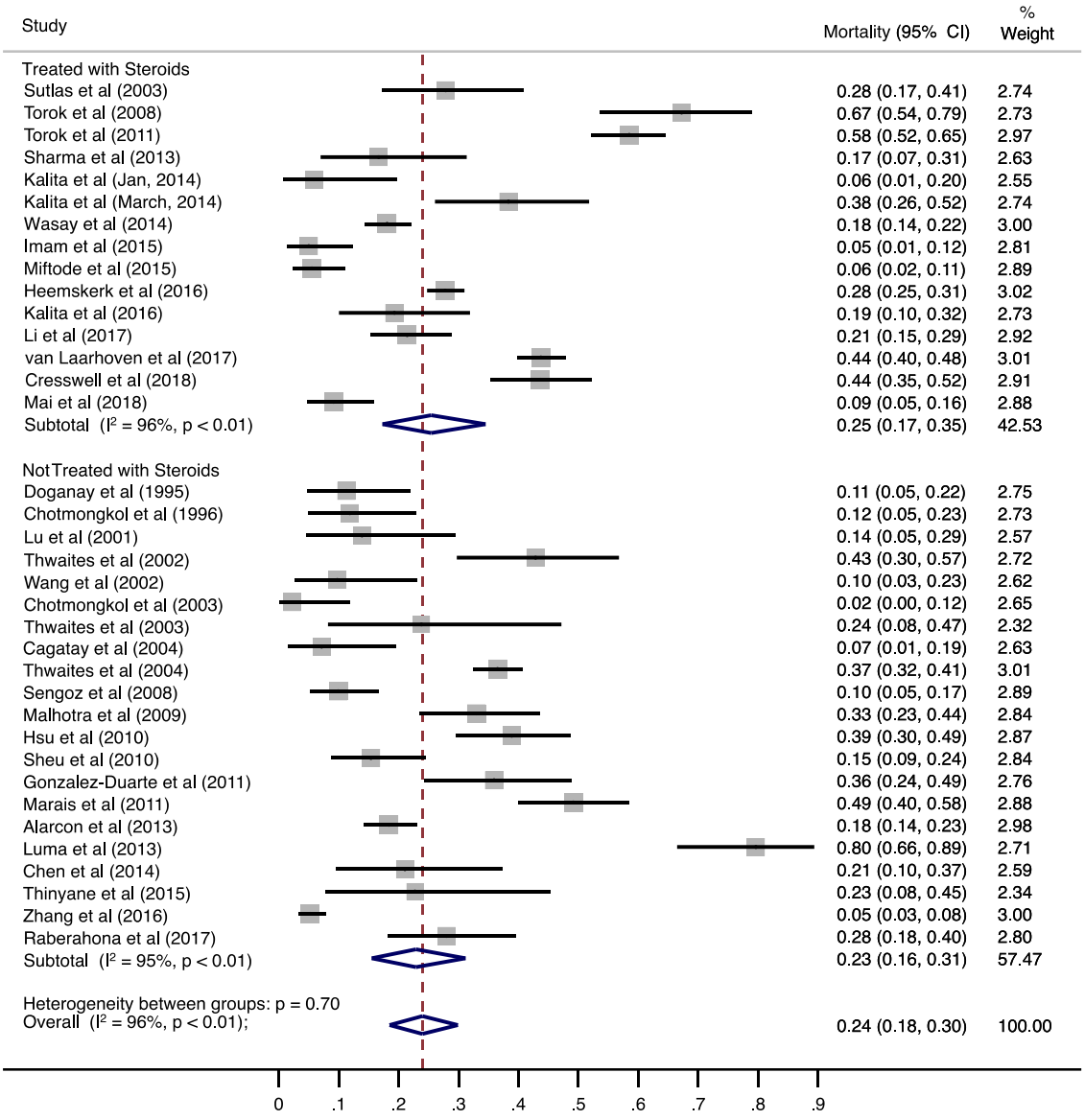
APPENDICES

Appendix A: Manuscript 1 Search Strategy

#	Database	Search term	Results
1	Medline	"TUBERCULOSIS, MENINGEAL"/	6850
2	Medline	((TB OR tubercul*) ADJ2 mening*).ti,ab	6694
3	Medline	((TB OR tubercul*) ADJ2 (brain OR cerebral OR neurological)).ti,ab	929
4	Medline	(1 OR 2 OR 3)	9169
5	Medline	exp MORTALITY/	343596
6	Medline	exp "TREATMENT OUTCOME"/	876952
7	Medline	exp "OUTCOME AND PROCESS ASSESSMENT (HEALTH CARE)"/	968126
8	Medline	exp "SURVIVAL ANALYSIS"/	257144
9	Medline	"TUBERCULOSIS, MENINGEAL"/mo	192
10	Medline	"TUBERCULOSIS, MENINGEAL"/co	1175
11	Medline	(sequalae OR sequelae OR complication* OR outcome* OR death* OR mortality OR survival).ti,ab	3381437
12	Medline	(5 OR 6 OR 7 OR 8 OR 9 OR 10 OR 11)	3965816
13	Medline	(4 AND 12)	2762
14	Medline	exp ADULT/	6572439
15	Medline	(adult* OR age OR aged).ti,ab	3017488
16	Medline	(14 OR 15)	7975296
17	Medline	(13 AND 16)	1593
18	EMBASE	"TUBERCULOUS MENINGITIS"/	6409
19	EMBASE	((TB OR tubercul*) ADJ2 mening*).ti,ab	5995
20	EMBASE	((TB OR tubercul*) ADJ2 (brain OR cerebral OR neurological)).ti,ab	831
21	EMBASE	(18 OR 19 OR 20)	8847
22	EMBASE	exp *MORTALITY/	129762
23	EMBASE	exp *"TREATMENT OUTCOME"/	69616
24	EMBASE	exp *SURVIVAL/	89772
25	EMBASE	*FATALITY/	4759
26	EMBASE	exp *COMPLICATION/	237190
27	EMBASE	*"ADVERSE OUTCOME"/	4524
28	EMBASE	(sequalae OR sequelae OR complication* OR outcome* OR death* OR mortality OR survival).ti,ab	4751087
29	EMBASE	(22 OR 23 OR 24 OR 25 OR 26 OR 27 OR 28)	4928804
30	EMBASE	(21 AND 29)	2534
31	EMBASE	exp *ADULT/	97301
32	EMBASE	(adult* OR age OR aged).ti,ab	4369238
33	EMBASE	(31 OR 32)	4393218
34	EMBASE	(30 AND 33)	969

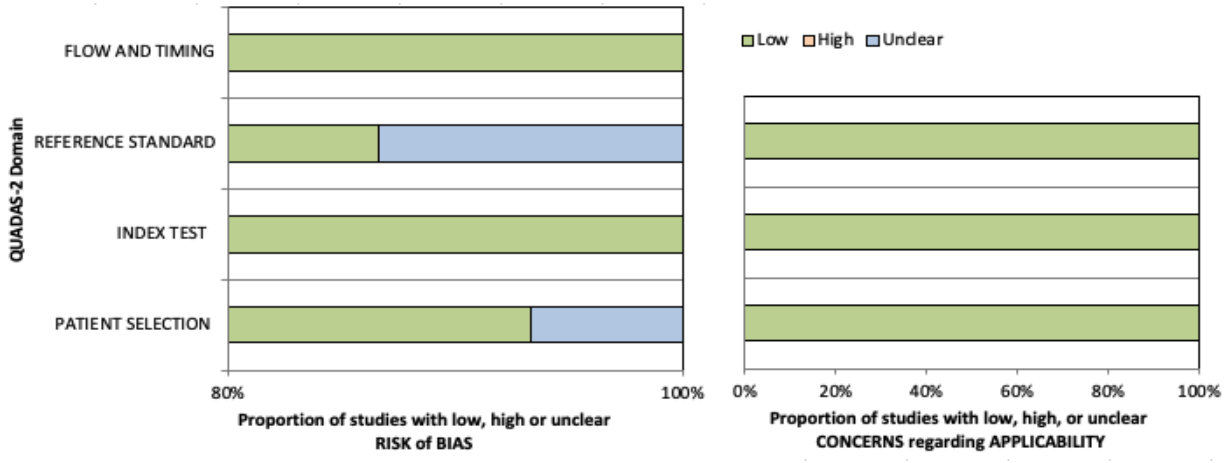
Appendix B: Tuberculous Meningitis Mortality by Steroid Use



Appendix C: Manuscript 2 Search Strategy

#1 Search tuberculosis meningitis Field: Title/Abstract
#2 Search “tuberculosis, meningeal”[MeSH]
#3 Search cerebral tuberculosis Field: Title/Abstract
#4 Search “brain tuberculosis” Field: Title/Abstract
#5 Search TBM Field: Title/Abstract
#6 Search ((((tuberculosis meningitis) OR “tuberculosis, meningeal”[MeSH Terms]) OR “cerebral tuberculosis“) OR “brain tuberculosis”) OR TBM
#7 Search “Diagnosis”[Majr]
#8 Search diagnosis or diagnostic Field: Title/Abstract
#9 Search “clinical scores” or “clinical scoring” Field: Title/Abstract
#10 Search “Research Design”[Mesh]
#11 Search predictor* or predictive Filters: Field: Title/Abstract
#12 Search “clinical predict*” Field: Title/Abstract
#13 Search “clinical feature*” Field: Title/Abstract
#14 Search (((#13 OR ((#12) OR ((#11) OR ((#10) OR ((#9) OR #8 OR #7 Filters: Humans
#15 Search #14 AND #6 Filters: Humans

Appendix D: QUADAS-2



Appendix E: Logistic Regression Coefficients with Average Intercept

Covariate	Odds Ratio (95% CI)	p-value
Intercept	0.53 (0.38-0.75)	<0.001
CSF WBC count, 100/mm^3	0.92 (0.91-0.94)	<0.001
Neutrophilic Dominance	4.55 (3.37-6.14)	<0.001
Lymphocytic Dominance	4.74 (3.74-6.01)	<0.001
CSF Glucose, 10 mg/dL	0.67 (0.64-0.71)	<0.001
Blood Glucose, 10 mg/dL	1.06 (1.04-1.09)	<0.001
CSF CrAg Positive	0.03 (0.02-0.05)	<0.001
Fever	1.06 (1.34-1.92)	<0.001
HIV-positive	1.14 (0.94-1.37)	0.176

Appendix F: Variable Importance in Random Forest Model

Covariate	Variable Importance*
CSF Glucose, mg/dL	367.27
CSF WBC count, cells/mm^3	310.12
CSF Protein, mg/dL	230.48
Blood Glucose, 10 mg/dL	153.98
CSF CrAg Positive	99.20
Brazil	35.25
CSF Lymphocytic Dominance	32.71
HIV-Positive	31.29
Vietnam	26.64
Fever	24.94
CSF Neutrophilic Dominance	22.46
Morocco	18.39
Indonesia	17.33
High TB Burden Country	15.83
Uganda	12.86
Romania	9.41
Botswana	9.18
Peru	5.68
South Africa	5.48

*Mean Decrease in Gini